Title: Hazards of diethyl phthalate (DEP) exposure: A systematic review of animal toxicology studies

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<u>**Abstract:**</u>

Background: Diethyl phthalate (DEP) is widely used in many commercially available products including plastics and personal care products. DEP has generally not been found to share the antiandrogenic mode of action that is common among other types of phthalates, but there is emerging evidence that DEP may be associated with other types of health effects.

Objective: To inform chemical risk assessment, we performed a systematic review to identify and characterize outcomes within six broad hazard categories (male reproductive, female reproductive, developmental, liver, kidney, and cancer) following exposure of nonhuman mammalian animals to DEP or its primary metabolite, monoethyl phthalate (MEP).

Methods: A literature search was conducted in five online scientific databases (PubMed, Web of Science, Toxline, Toxic Substances Control Act Test Submissions, and Toxcenter) augmented by review of online regulatory sources as well as forward and backward searches. Studies were selected for inclusion using PECO (Population, Exposure, Comparator, Outcome) criteria. Studies were evaluated using criteria defined a priori for reporting quality, risk of bias, and sensitivity using a domain-based approach. Evidence was synthesized by outcome and life stage of exposure, and strength of evidence was summarized into categories of *robust*, *moderate*, *slight*, *indeterminate*, or *compelling evidence of no effect*, using a structured framework.

Results: Thirty experimental studies in animals were included in this analysis. Although no effects on androgen-dependent male reproductive development were observed following gestational exposure to DEP, there was evidence including effects on sperm following peripubertal and adult exposures, and the overall evidence for male reproductive effects was considered *moderate*. There was *moderate* evidence that DEP exposure can lead to developmental effects, with the major effect being reduced postnatal growth following gestational or early postnatal exposure; this generally occurred at doses associated with maternal effects, consistent with the observation that DEP is not a potent developmental toxicant. The evidence for liver effects was considered *moderate* based on consistent changes in relative liver weight at higher dose levels; histopathological and biochemical changes indicative of hepatic effects were also observed, but primarily in studies that had significant concerns for risk of bias and sensitivity. The evidence for female reproductive effects was considered *slight* based on few reports of significant effects on maternal body weight gain, organ weight changes, and pregnancy outcomes. Evidence for cancer and effects on kidney were judged to be *indeterminate* based on limited evidence (i.e., a single two-year cancer bioassay) and inconsistent findings, respectively.

Conclusions: These results suggest that DEP exposure induces androgen-independent male reproductive toxicity (i.e., sperm effects) as well as developmental toxicity and hepatic effects, with some evidence of female reproductive toxicity. More research is warranted to fully evaluate these outcomes and strengthen confidence in this database.

Highlights:

- Gestational exposure to DEP did not affect testosterone production or cause phthalate syndrome in rats.
- Effects of DEP on sperm may be consistent with the androgen-independent mode of action for phthalates.
- DEP is not a potent developmental toxicant, although skeletal variations and decreased growth were observed.
- Low dose studies observed large magnitudes of effect but significant concerns for bias were identified.

1. INTRODUCTION:

Diethyl phthalate (DEP), a colorless, odorless oily substance, is used to improve the performance and durability of many products [ADDIN EN.CITE <EndNote><Cite><Author>Consumer Product Safety Commission</Author><Year>2011</Year><RecNum>82</RecNum><DisplayText>(Consumer Product Safety Commission 2011; World Health Organization 2003)</DisplayText><record><rec-number>82</rec-number><foreign-keys><key app="EN" db-id="rpv9f5v5cfrzxgesv5bv9sdn0e9dz2vdpzwx" timestamp="1544715975">82</key></foreign-keys><ref-type name="Report">27</ref-type><contributors><authors><author>Consumer Product Safety Commission,</author></authors></contributors><titles><title>Final toxicity review for diethyl phthalate
(DEP)</title></titles><dates><year>2011</year></dates><label>3685047</label><urls><related-urls><url>https://www.cpsc.gov/s3fs-public/dep.pdf</url></related-urls></urls><language>English</language></record></Cite><Cite><Author>World Health Organization</Author><Year>2003</Year><RecNum>83</RecNum><record><rec-number>83</rec-number><foreign-keys><key app="EN" db-id="rpv9f5v5cfrzxgesv5bv9sdn0e9dz2vdpzwx" timestamp="1544717014">83</key></foreign-keys><ref-type name="Report">27</ref-type><contributors><authors><author>World Health Organization,</author></authors></contributors><titles><title>Concise International Chemical Assessment Document 52: Diethyl
phthalate</title></titles><volume>CICAD</volume><dates><year>2003</year></dates><pub-location>Geneva</pub-location><isbn>RISKLINE/2003120009</isbn><label>1313351</label><urls><related-urls><url>http://www.who.int/ipcs/publications/cicad/en/cicad52.pdf</url></related-urls></urls><language>English</language></record></Cite></EndNote>]. As a plasticizer, it is added to plastic polymers to help maintain flexibility. It has been used in a variety of products including plastic films, rubber, tape, toothbrushes, automotive components, tool handles and toys. In addition to plastics, DEP is present in a wide range of personal care products (e.g., cosmetics, perfume, hair spray, nail polish, soap, detergent, and lotions), industrial materials (e.g., rocket propellant, dyes, packaging, sealants and lubricants), and medical products (e.g., enteric coatings on tablets and in dental impression materials).

Phthalates including DEP are not covalently bound to products, and therefore are readily released into the environment where they may be absorbed orally, by inhalation, or dermally. Following oral exposure in rats, DEP primarily locates to the kidneys and liver followed by deposition in fat [ADDIN EN.CITE <EndNote><Cite><Author>Singh</Author><Year>1975</Year><RecNum>13</RecNum><DisplayText>(Singh et al. 1975)</DisplayText><record><rec-number>13</rec-number><foreign-keys><key app="EN" db-id="rpv9f5v5cfrzxgesv5bv9sdn0e9dz2vdpzwx" timestamp="0">13</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Singh, A. R.</author><author>Lawrence, W. H.</author><author>Autian, J.</author></authors></contributors><titles><title>Maternal-fetal transfer of 14C-di-2-ethylhexyl phthalate and 14C-diethyl phthalate in rats</title><secondary-title>Journal of Pharmaceutical Sciences</secondary-title><alt-title>J Pharm Sci</alt-title><short-title>Journal of Pharmaceutical Sciences</short-title></titles><periodical><full-title>Journal of Pharmaceutical Sciences</full-title><abbr-1>J Pharm Sci</abbr-1></periodical><alt-periodical><full-title>Journal of Pharmaceutical Sciences</full-title><abbr-1>J Pharm Sci</abbr-1></alt-periodical><pages>1347-

1350</pages><volume>64</volume><number>8</number><edition>NOT IN
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urls></urls><custom1>false</custom1><electronic-resource-
num>10.1002/jps.2600640819</electronic-resource-
num><language>English</language></record></Cite></EndNote>]. DEP is rapidly metabolized into the active metabolite monoethyl phthalate (MEP), which is ultimately excreted into the urine and serves as a biomarker of DEP exposure. Exposure assessment data from the National Health and Nutrition Examination Survey (NHANES) show that MEP was detected in the urine of at least 98% of participants in the US general population in each survey cycle between 2001 and 2010; urinary concentrations of MEP declined significantly over that time, with a more pronounced trend towards decreased urinary MEP in adults and adolescents compared to children, perhaps reflecting a trend towards decreased use of DEP in personal care products [ADDIN EN.CITE
<EndNote><Cite><Author>Zota</Author><Year>2014</Year><RecNum>40</RecNum><DisplayText>(Zo
ta et al. 2014)</DisplayText><record><rec-number>40</rec-number><foreign-keys><key app="EN" db-
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R.</author><author>Calafat, A. M.</author><author>Woodruff, T.
J.</author></authors></contributors><titles><title>Temporal trends in phthalate exposures: findings
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241</pages><volume>122</volume><number>3</number><dates><year>2014</year></dates><isbn>I
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2205,2206,2207,2245,2247,2320</url></related-urls></urls><custom1>true</custom1><electronic-
resource-num>10.1289/ehp.1306681</electronic-resource-
num><language>English</language></record></Cite></EndNote>]. Despite this trend, MEP levels tended to remain higher compared to other phthalate metabolites in urine across age groups in this study [ADDIN EN.CITE
<EndNote><Cite><Author>Zota</Author><Year>2014</Year><RecNum>40</RecNum><DisplayText>(Zo
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R.</author><author>Calafat, A. M.</author><author>Woodruff, T.
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title><short-title>Environmental Health Perspectives</short-title></titles><pages>235-
241</pages><volume>122</volume><number>3</number><dates><year>2014</year></dates><isbn>I
SSN 0091-6765
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num><label>2241689</label><urls><related-urls><url>https://heronet.epa.gov/heronet/index.cfm/reference/download/reference_id/2241689C3 - 2205,2206,2207,2245,2247,2320</url></related-urls></urls><custom1>true</custom1><electronic-resource-num>10.1289/ehp.1306681</electronic-resource-num><language>English</language></record></Cite></EndNote>]. Other analyses of biomonitoring data have similarly found that DEP is among the highest phthalate exposures for women of childbearing age, with personal care products being a major source [ADDIN EN.CITE <EndNote><Cite><Author>National Research Council</Author><Year>2008</Year><RecNum>52</RecNum><DisplayText>(Consumer Product Safety Commission 2014; National Research Council 2008)</DisplayText><record><rec-number>52</rec-number><foreign-keys><key app="EN" db-id="rpv9f5v5cfrzxgesv5bv9sdn0e9dz2vdpzwx" timestamp="0">52</key></foreign-keys><ref-type name="Book Section">5</ref-type><contributors><authors><author>National Research Council,</author></authors></contributors><titles><secondary-title>Phthalates and Cumulative Risk Assessment: The Tasks Ahead</secondary-title></titles><dates><year>2008</year></dates><pub-location>Washington (DC)</pub-location><publisher>National Academies Press (US)
Copyright 2008 by the National Academy of Sciences. All rights reserved.</publisher><accession-num>25009926</accession-num><urls></urls><electronic-resource-num>10.17226/12528</electronic-resource-num><language>eng</language></record></Cite><Cite><Author>Consumer Product Safety Commission</Author><Year>2014</Year><RecNum>41</RecNum><record><rec-number>41</rec-number><foreign-keys><key app="EN" db-id="rpv9f5v5cfrzxgesv5bv9sdn0e9dz2vdpzwx" timestamp="0">41</key></foreign-keys><ref-type name="Report">27</ref-type><contributors><authors><author>Consumer Product Safety Commission,</author></authors></contributors><titles><title>Chronic Hazard Advisory Panel on phthalates and phthalate alternatives (with appendices)</title></titles><dates><year>2014</year></dates><pub-location>Bethesda, MD</pub-location><publisher>U.S. Consumer Product Safety Commission, Directorate for Health Sciences</publisher><label>2439960</label><urls><related-urls><url>https://heronet.epa.gov/heronet/index.cfm/reference/download/reference_id/2439960C3 - 1097,2206,2207,2247,2320</url></related-urls></urls><language>English</language><modified-date>Chronic Hazard Advisory Panel on Phthalates and Phthalate Alternatives</modified-date></record></Cite></EndNote>].

Unlike multiple other phthalates, DEP has not been found to inhibit fetal testosterone production [ADDIN EN.CITE <EndNote><Cite><Author>Consumer Product Safety Commission</Author><Year>2014</Year><RecNum>41</RecNum><DisplayText>(Consumer Product Safety Commission 2014)</DisplayText><record><rec-number>41</rec-number><foreign-keys><key app="EN" db-id="rpv9f5v5cfrzxgesv5bv9sdn0e9dz2vdpzwx" timestamp="0">41</key></foreign-keys><ref-type name="Report">27</ref-type><contributors><authors><author>Consumer Product Safety Commission,</author></authors></contributors><titles><title>Chronic Hazard Advisory Panel on phthalates and phthalate alternatives (with appendices)</title></titles><dates><year>2014</year></dates><pub-location>Bethesda, MD</pub-location><publisher>U.S. Consumer Product Safety Commission, Directorate for Health Sciences</publisher><label>2439960</label><urls><related-urls><url>https://heronet.epa.gov/heronet/index.cfm/reference/download/reference_id/2439960C3 -

1097,2206,2207,2247,2320</url></related-urls></urls><language>English</language><modified-date>Chronic Hazard Advisory Panel on Phthalates and Phthalate Alternatives</modified-date></record></Cite></EndNote>], which is one of the major mechanisms underlying the "phthalate syndrome" phenotype that is observed in male rats following gestational exposure to phthalates. Phthalate syndrome is characterized by a spectrum of effects including underdevelopment of male reproductive organs, decreased anogenital distance (AGD), female-like nipple retention, cryptorchidism, and germ cell toxicity [ADDIN EN.CITE <EndNote><Cite><Author>National Research Council</Author><Year>2008</Year><RecNum>52</RecNum><DisplayText>(Consumer Product Safety Commission 2014; National Research Council 2008)</DisplayText><record><rec-number>52</rec-number><foreign-keys><key app="EN" db-id="rpv9f5v5cfrzxgesv5bv9sdn0e9dz2vdpzwx" timestamp="0">52</key></foreign-keys><ref-type name="Book Section">5</ref-type><contributors><authors><author>National Research Council,</author></authors></contributors><titles><secondary-title>Phthalates and Cumulative Risk Assessment: The Tasks Ahead</secondary-title></titles><dates><year>2008</year></dates><pub-location>Washington (DC)</pub-location><publisher>National Academies Press (US)
Copyright 2008 by the National Academy of Sciences. All rights reserved.</publisher><accession-num>25009926</accession-num><urls></urls><electronic-resource-num>10.17226/12528</electronic-resource-num><language>eng</language></record></Cite><Cite><Author>Consumer Product Safety Commission</Author><Year>2014</Year><RecNum>41</RecNum><record><rec-number>41</rec-number><foreign-keys><key app="EN" db-id="rpv9f5v5cfrzxgesv5bv9sdn0e9dz2vdpzwx" timestamp="0">41</key></foreign-keys><ref-type name="Report">27</ref-type><contributors><authors><author>Consumer Product Safety Commission,</author></authors></contributors><titles><title>Chronic Hazard Advisory Panel on phthalates and phthalate alternatives (with appendices)</title></titles><dates><year>2014</year></dates><pub-location>Bethesda, MD</pub-location><publisher>U.S. Consumer Product Safety Commission, Directorate for Health Sciences</publisher><label>2439960</label><urls><related-urls><url>https://heronet.epa.gov/heronet/index.cfm/reference/download/reference_id/2439960C3 - 1097,2206,2207,2247,2320</url></related-urls></urls><language>English</language><modified-date>Chronic Hazard Advisory Panel on Phthalates and Phthalate Alternatives</modified-date></record></Cite></EndNote>]. These effects are driven not only by a phthalate-induced decrease in testicular testosterone production, but also by decreased production of insulin-like-3 hormone and disrupted seminiferous cord formation, Sertoli cells, and germ cell development, which occur independently of changes in androgen production [ADDIN EN.CITE ADDIN EN.CITE.DATA]. A recent review by a Chronic Hazard Advisory Panel (CHAP), which focused on phthalates and phthalate alternatives used in children's toys and child care products, found that DEP does not cause phthalate syndrome in rats, although decreased testosterone and effects on sperm were observed in some studies in adult and peripubertal animals, and there were some associations between urinary MEP and male reproductive outcomes in humans [ADDIN EN.CITE <EndNote><Cite><Author>Consumer Product Safety Commission</Author><Year>2014</Year><RecNum>41</RecNum><DisplayText>(Consumer Product Safety Commission 2014)</DisplayText><record><rec-number>41</rec-number><foreign-keys><key app="EN" db-id="rpv9f5v5cfrzxgesv5bv9sdn0e9dz2vdpzwx" timestamp="0">41</key></foreign-keys><ref-type name="Report">27</ref-type><contributors><authors><author>Consumer Product Safety Commission,</author></authors></contributors><titles><title>Chronic Hazard Advisory Panel on

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The aim of this systematic review is to characterize the range of health outcomes that are associated with DEP exposure in animal toxicology studies, including effects in both males and females, and for all life stages of exposure. This systematic review focuses on six broad hazard categories (male reproductive, female reproductive, developmental, liver, kidney, cancer) that have been commonly associated with phthalate exposure. The results provide a more comprehensive understanding of the effects of DEP exposure and helps to identify gaps in the currently available literature.

2. METHODS:

Several individual systematic reviews of epidemiological and animal toxicological studies, were published describing health effects following exposure to phthalates [ADDIN EN.CITE ADDIN EN.CITE.DATA]. This systematic review continues the evaluation of health effects after exposure to phthalates. The literature searches and screening, study evaluation, data extraction, and evidence synthesis methods are described in detail in the systematic review protocol (provided as a supplementary file) and summarized here. The systematic review protocol also provides detailed definitions for the terminology used to describe study evaluation and evidence synthesis, which are summarized in Figure 1. For easier reference, these definitions and key methods from the protocol related to study evaluation and evidence synthesis are also summarized in a separate supplementary file ("key methods supplement").

2.1. Literature searches and screening

A literature search was conducted in five online scientific databases [PubMed, Web of Science, Toxline, TSCATS and Toxcenter], using search terms designed to capture all potentially pertinent studies. Initial database searches were conducted in March 2012, with updates performed every 6-12 months through July 2017 (see protocol Section 3). The results of this literature search were supplemented by forward and backward searches, searching citations from key references, manual search of citations from key regulatory documents, and by addition of references that had been previously identified from an earlier DEP review effort and added to EPA's Health and Environmental Research Online (HERO) database ([HYPERLINK "https://hero.epa.gov/hero/index.cfm/project/page/project_id/1097"]).

A PECO (Population, Exposure, Comparator, Outcome) was developed to frame the research question and guide the screening of relevant studies. The PECO identifies the following as the inclusion criteria for the systematic review of DEP animal toxicology studies (see protocol Section 2 for the full PECO):

- Population: Nonhuman mammalian animal species (whole organism) of any life stage.
- Exposure: Any administered dose of DEP or MEP as singular compounds, via oral, dermal, or inhalation routes of exposure.
- Comparator: Exposure to vehicle-only or untreated control
- Outcome: Any examination of male reproductive, female reproductive, developmental, liver, kidney, or cancer outcomes.

Title/abstract and full text screening were performed by two reviewers, and all identified animal toxicology studies underwent full-text screening to determine compliance with the PECO. Peer-reviewed studies that contained original data and complied with the PECO were selected for inclusion and were moved forward for study evaluation. Studies providing supporting health effects data (e.g. mechanistic, genotoxic, or toxicokinetic studies) were also compiled in HERO and annotated during the screening process.

2.2. Study evaluation

For each study selected for inclusion, the quality and informativeness of the evidence was rated by evaluating domains related to reporting quality, risk of bias, and sensitivity (see protocol Section 4; abbreviated version available in the key methods supplement). Evaluations first considered reporting quality, which refers to whether the study has reported sufficient details to conduct a risk of bias and sensitivity analysis; if a study does not report critical information (e.g. species, test article name) it may be excluded from further consideration. Risk of bias, sometimes referred to as internal validity, is the extent to which the design or conduct of a study may alter the ability to provide accurate (unbiased) evidence to support the relationship between exposure and effects [ADDIN EN.CITE <EndNote><Cite><Author>Higgins</Author><Year>2011</Year><RecNum>59</RecNum><DisplayText>(Higgins 2011)</DisplayText><record><rec-number>59</rec-number><foreign-keys><key app="EN" db-id="rpv9f5v5cfrzxgesv5bv9sdn0e9dz2vdpzwx" timestamp="0">59</key></foreign-keys><ref-type name="Electronic Book">44</ref-type><contributors><authors><author>Higgins, JPTG</author></authors></contributors><titles><title>Cochrane handbook for systtematic reviews of interventions </title><secondary-title>Chapter 8: Assessing risk of bias in included studies. </secondary-title></titles><num-vols>5.1.0</num-vols><dates><year>2011</year></dates><publisher>The Cochrane collaboration [updated March 2011]</publisher><urls></urls></record></Cite></EndNote>].

Sensitivity refers to the extent to which a study is likely to detect a true effect caused by exposure [ADDIN EN.CITE
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<EndNote><Cite><Author>Higgins</Author><Year>2011</Year><RecNum>59</RecNum><DisplayText>(Higgins 2011)</DisplayText><record><rec-number>59</rec-number><foreign-keys><key app="EN" db-id="rpv9f5v5cfrzxgesv5bv9sdn0e9dz2vdpzwx" timestamp="0">59</key></foreign-keys><ref-type name="Electronic Book">44</ref-type><contributors><authors><author>Higgins, JPTG</author></authors></contributors><titles><title>Cochrane handbook for systtematic reviews of interventions </title><secondary-title>Chapter 8: Assessing risk of bias in included studies. </secondary-title></titles><num-vols>5.1.0</num-vols><dates><year>2011</year></dates><publisher>The Cochrane collaboration [updated March 2011]</publisher><urls></urls></record></Cite></EndNote>].
Sensitivity refers to the extent to which a study is likely to detect a true effect caused by exposure [ADDIN EN.CITE
<EndNote><Cite><Author>Cooper</Author><Year>2016</Year><RecNum>46</RecNum><DisplayText>(Cooper et al. 2016)</DisplayText><record><rec-number>46</rec-number><foreign-keys><key app="EN" db-id="rpv9f5v5cfrzxgesv5bv9sdn0e9dz2vdpzwx" timestamp="0">46</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Cooper, G.</author><author>Lunn, R.</author><author>Agerstrand, M.</author><author>Glenn, B.</author><author>Kraft, A.</author><author>Luke, A.</author><author>Ratcliffe, J.</author></authors></contributors><titles><title>Study sensitivity: Evaluating the ability to detect effects in systematic reviews of chemical exposures</title><secondary-title>Environment International</secondary-title><alt-title>Environ Int</alt-title><short-title>Environment International</short-title></titles><periodical><full-title>Environ Int</full-title><abbr-1>Environment international</abbr-1></periodical><alt-periodical><full-title>Environ Int</full-title><abbr-1>Environment international</abbr-1></alt-periodical><pages>605-610</pages><volume>92-93</volume><dates><year>2016</year></dates><isbn>ISSN 0160-4120
EISSN 1873-

6750</isbn><label>3121908</label><urls><related-urls><url>https://heronet.epa.gov/heronet/index.cfm/reference/download/reference_id/3121908C3 - 1723,2245,2361,2489,2529,2558,2561,2562,2563,2564,2566,2567,2665</url></related-urls></urls><custom1>true</custom1><electronic-resource-num>10.1016/j.envint.2016.03.017</electronic-resource-num><language>English</language></record></Cite></EndNote>].

All study evaluation ratings are documented and publicly available in EPA's version of Health Assessment Workspace Collaborative (HAWC), a free and open source web-based software application ([HYPERLINK "https://hawcprd.epa.gov/assessment/552/"]). Study evaluation was conducted in the following domains: reporting quality; allocation; observational bias/blinding; confounding; selective reporting and attrition; chemical administration and characterization; exposure timing, frequency and duration; endpoint sensitivity and specificity; and results presentation. For each domain, core questions and basic considerations provided guidance on how a reviewer might evaluate and judge a study for that domain (see Table 9 of the protocol or Table A of the key methods supplement).

At least two reviewers independently assessed each study, and any conflicts were resolved through discussion among reviewers or other technical experts. When information needed for the evaluation was missing from a key study, an attempt was made to contact the study authors for clarification. All communication with study authors was documented and is available in HERO (tagged as Personal Correspondence with Authors) and was annotated in HAWC whenever it was used to inform a study evaluation.

For each study, in each evaluation domain, reviewers reached a consensus on a rating of *Good*, *Adequate*, *Deficient*, or *Critically Deficient*. These individual ratings were then combined to reach an overall study confidence classification of *High*, *Medium*, *Low*, or *Uninformative*. The evaluation process was performed separately for each outcome reported in a study, as the utility of a study may vary for different outcomes.

2.3. Data extraction

Data from included studies were extracted into HAWC ([HYPERLINK "https://hawcprd.epa.gov/assessment/552/"]). Dose levels are presented as mg/kg-day.[ADDIN EN.CITE <EndNote><Cite><Author>EPA</Author><Year>1988</Year><RecNum>55</RecNum><DisplayText>(US EPA 1988)</DisplayText><record><rec-number>55</rec-number><foreign-keys><key app="EN" db-id="rpv9f5v5cfrzxgesv5bv9sdn0e9dz2vdpzwx" timestamp="0">55</key></foreign-keys><ref-type name="Government Document">46</ref-type><contributors><authors><author>US EPA,</author></authors><secondary-authors><author>Office of Research and Development, Office of Health and Environmental Assessment</author></secondary-authors></contributors><titles><title>Recommedations for and documentation of biological values for use in risk assessment</title></titles><dates><year>1988</year></dates><pub-location>Cincinnati, OH</pub-location><isbn>EPA/600/6-87/008</isbn><urls></urls><custom1>US Environmental Protection Agency</custom1></record></Cite></EndNote>] For dietary exposure studies, dose conversions to mg/kg-day were made using US EPA default food or water consumption rates and body weights for the species/strain and sex of the animal of interest [ADDIN EN.CITE <EndNote><Cite><Author>EPA</Author><Year>1988</Year><RecNum>55</RecNum><DisplayText>(US

EPA 1988)</DisplayText><record><rec-number>55</rec-number><foreign-keys><key app="EN" db-id="rpv9f5v5cfrzxgesv5bv9sdn0e9dz2vdpzwx" timestamp="0">55</key></foreign-keys><ref-type name="Government Document">46</ref-type><contributors><authors><author>US EPA,</author></authors><secondary-authors><author>Office of Research and Development, Office of Health and Environmental Assessment</author></secondary-authors></contributors><titles><title>Recommedations for and documentation of biological values for use in risk assessment</title></titles><dates><year>1988</year></dates><pub-location>Cincinnati, OH</pub-location><isbn>EPA/600/6-87/008</isbn><urls></urls><custom1>US Environmental Protection Agency</custom1></record></Cite></EndNote>].

2.4. Evidence synthesis

For each outcome, the available evidence from the included animal studies was synthesized using a narrative approach, using the following considerations to articulate the strengths and weaknesses of the available evidence [adapted from [ADDIN EN.CITE <EndNote><Cite><Author>Hill</Author><Year>1965</Year><RecNum>4</RecNum><DisplayText>(Hill 1965)</DisplayText><record><rec-number>4</rec-number><foreign-keys><key app="EN" db-id="rpv9f5v5cfrzxgesv5bv9sdn0e9dz2vdpzwx" timestamp="0">4</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Hill, A. B.</author></authors></contributors><titles><title>The environment and disease: Association or causation?</title><secondary-title>Proceedings of the Royal Society of Medicine</secondary-title><alt-title>Proc R Soc Med</alt-title><short-title>Proceedings of the Royal Society of Medicine</short-title></titles><pages>295-300</pages><volume>58</volume><number>5</number><dates><year>1965</year></dates><isbn>ISSN 0035-9157</isbn><accession-num>14283879</accession-num><label>71664</label><urls><related-urls><url>https://heronet.epa.gov/heronet/index.cfm/reference/download/reference_id/71664C3 -14,15,16,31,32,36,40,44,49,290,325,420,1164,1542,1543,1574,1628,1676,1678,1723,1826,1836,1852,1853,1891,1892,2186,2211,2212,2216,2251,2296,2302,2321,2338,2361,2375,2489,2526,2532,2571,2573,2575,2582,2583,2584,2585,2587,2588,2673</url></related-urls></urls><custom1>true</custom1><language>English</language></record></Cite></EndNote>]]: consistency, biological gradient (dose-response), strength (effect magnitude) and precision, biological plausibility, and coherence. When possible, an effort was made to evaluate the data according to the age and developmental stage of exposure to account for life stage-specific windows of susceptibility, as recommended by EPA's Framework for assessing health risk of environmental exposures to children [HYPERLINK \l "_ENREF_65" \o "US EPA, 2006 #64"] and by [HYPERLINK \l "_ENREF_27" \o "Makris, 2008 #79"]. When available, informative mechanistic data were used to augment the qualitative syntheses.

Based on this synthesis, each outcome was assigned a strength of evidence conclusion of *Robust, Moderate, Slight, Indeterminate*, or *Compelling evidence of no effect* (see definitions of these terms in Table 12 of the protocol or Table B of the key methods supplement). *Robust* and *Moderate* describe evidence that supports a hazard, differentiated by the quantity and quality of information available to rule out alternative explanations for the results (*Robust* describes an evidence base for which there is reasonable confidence that results are not due to chance, bias, or confounding; whereas *Moderate* describes an evidence base that has greater uncertainty, e.g. due to a smaller number of studies or some heterogeneity in results). *Slight* evidence includes situations in which there is some evidence that supports a hazard, but a conclusion of *Moderate* does not apply. *Indeterminate* describes a situation where the evidence is limited or inconsistent and cannot provide a basis for making a conclusion in either direction, or if the available studies are largely null but do not reach the level required to conclude there is compelling evidence of no effect. *Compelling evidence of no effect* represents a situation where extensive evidence across a range of populations and exposures identified no association between exposure and hazard. The ratings for individual outcomes were then summarized into an overall strength of evidence conclusion for each of the six hazards (male reproductive, female reproductive, developmental, liver, kidney, cancer). Rationales for strength of evidence conclusions are presented in evidence profile tables using a structured format based on the Grading of Recommendations Assessment, Development and Evaluation (GRADE) approach for evaluating certainty in the evidence [ADDIN EN.CITE ADDIN EN.CITE.DATA].

3. RESULTS

3.1. Study selection:

Literature search and screening results are summarized in Figure 2. The literature search retrieved a total of 1,679 unique records for DEP, of which 73 were identified as animal health effect studies. Of the animal health effect studies, 40 were excluded for not meeting PECO criteria. Common reasons for exclusion included skin and eye irritation studies which were not considered in this evaluation and the use of non-mammalian species. Three pairs of the included studies were found to be multiple publications of the same data [[HYPERLINK \l "_ENREF_17" \o "Hazelton Laboratories America Inc, 1983 #12"] and [HYPERLINK \l "_ENREF_15" \o "Hardin, 1987 #3"], [HYPERLINK \l "_ENREF_25" \o "Lamb, 1987 #1"] and [HYPERLINK \l "_ENREF_54" \o "RTI International, 1984 #28"], and [HYPERLINK \l "_ENREF_8" \o "Field, 1993 #11"] and [HYPERLINK \l "_ENREF_34" \o "NTP, 1988 #29"]] and were considered thereafter to be a single study [cited here as [HYPERLINK \l "_ENREF_15" \o "Hardin, 1987 #3"], [HYPERLINK \l "_ENREF_54" \o "RTI International, 1984 #28"], and [HYPERLINK \l "_ENREF_34" \o "NTP, 1988 #29"]]. Therefore, a total of 30 studies were included this analysis (Table 1).

Summary of included studies:

The included studies are summarized in Table 1. The database of DEP studies is diverse and consists of multigenerational studies, gestational exposure studies, and studies in peripubertal or adult animals. All studies were either oral or dermal exposures conducted in rats, rabbits or mice. [HYPERLINK \l "_ENREF_23" \o "Kwack, 2009 #14"] [HYPERLINK \l "_ENREF_19" \o "Kwack, 2009 #14"]exposed animals to either DEP or MEP, and all other studies used DEP.

Eight studies assessed multigenerational effects of DEP exposure. Of these, reproductive and developmental effects were evaluated in the continuous breeding study in mice by [HYPERLINK \l "_ENREF_54" \o "RTI International, 1984 #28"] and in the two-generation reproduction study in rats by [HYPERLINK \l "_ENREF_10" \o "Fujii, 2005 #22"], which exposed animals beginning prior to the mating of F0 parental animals and continuing through the weaning of F2 offspring. Four multigenerational studies report data from the same group of animals exposed to a low dose of DEP (approximately 2.85 mg/kg-day) from the F0 to F2 generations, focusing on either hepatic toxicity [ADDIN EN.CITE ADDIN EN.CITE.DATA] or reproductive toxicity [ADDIN EN.CITE <EndNote><Cite><Author>Pereira</Author><Year>2007</Year><RecNum>38</RecNum><DisplayText>(Pereira et al. 2007a)</DisplayText><record><rec-number>38</rec-number><foreign-keys><key app="EN" db-id="rpv9f5v5cfrzxgesv5bv9sdn0e9dz2vdpzwx" timestamp="0">38</key></foreign-

keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Pereira, C.</author><author>Mapuskar, K.</author><author>Rao, C. V.</author></authors></contributors><titles><title>Reproductive failure associated with chronic interactive mixture toxicity of diethyl phthalate and Clophen A60 after gestational and lactational exposure over two generations in Wistar rats</title><secondary-title>Toxicology International</secondary-title></titles><periodical><full-title>Toxicology International</full-title></periodical><pages>111-122</pages><volume>14</volume><number>2</number><dates><year>2007</year></dates><label>1325854</label><urls><related-urls><url>https://heronet.epa.gov/heronet/index.cfm/reference/download/reference_id/1325854C3 - 1097,2305,2344</url></related-urls></urls><custom1>true</custom1><language>English</language></record></Cite></EndNote>].
The remaining two multigenerational studies [ADDIN EN.CITE ADDIN EN.CITE.DATA] exposed F1 female rats to a low dose (0.173 mg/kg-day) of DEP beginning at postnatal day (PND) 1 via lactation, and continuing through PND 181 via oral gavage; [HYPERLINK \l "_ENREF_29" \o "Manservisi, 2015 #48"] reported on mammary gland effects and fertility in F1 females and growth and survival of F2 offspring, and [HYPERLINK \l "_ENREF_21" \o "Hu, 2016 #47"] reported body weight data for a subset of these same F1 female rats at PNDs 62 and 181.

Effects in developing animals were also evaluated in studies that exposed animals during gestation and/or early postnatal life. Of these, the studies by [HYPERLINK \l "_ENREF_12" \o "Gray, 2000 #10"], [HYPERLINK \l "_ENREF_20" \o "Howdeshell, 2008 #8"], [HYPERLINK \l "_ENREF_11" \o "Furr, 2014 #42"], and [HYPERLINK \l "_ENREF_26" \o "Liu, 2005 #80"] exposed rats during late gestation [gestation day (GD) 14 – PND 3, GD 8-18, GD 14 – 18, and GD 12-19, respectively] and focused on male reproductive development and phthalate syndrome effects. These exposures coincide with the critical window of male sexual differentiation (~GD 14-18), which is known to be the sensitive window of exposure for the induction of phthalate syndrome. [HYPERLINK \l "_ENREF_15" \o "Hardin, 1987 #3"] evaluated fetal survival and growth in mice exposed to a single high dose of DEP from gestation day (GD) 6-13. [HYPERLINK \l "_ENREF_34" \o "NTP, 1988 #29"] and [HYPERLINK \l "_ENREF_49" \o "Procter & Gamble, 1994 #32"] evaluated fetal survival, growth, and structural alterations in rats and rabbits following exposure from GD 6-15 and 6-18, respectively. These developmental exposure studies also provided relevant data on maternal reproductive endpoints (e.g. maternal body weight gain, pregnancy outcomes) in addition to effects on the developing animals.

The remaining studies evaluated rats or mice following peripubertal or adult exposure. [HYPERLINK \l "_ENREF_57" \o "Shiraishi, 2006 #30"] and [HYPERLINK \l "_ENREF_36" \o "Oishi, 1980 #2"] both focused primarily on reproductive outcomes; [HYPERLINK \l "_ENREF_36" \o "Oishi, 1980 #2"] evaluated testosterone and sperm parameters in male rats, whereas [HYPERLINK \l "_ENREF_57" \o "Shiraishi, 2006 #30"] evaluated effects in both male and female rats including hormone levels, sperm parameters, and estrous cyclicity. [HYPERLINK \l "_ENREF_24" \o "Kwack, 2010 #87"] and [HYPERLINK \l "_ENREF_23" \o "Kwack, 2009 #14"] both evaluated general toxicity (organ weights, serum biochemistry, urinalysis) in DEP- and MEP-exposed rats, with a sperm evaluation also conducted in [HYPERLINK \l "_ENREF_23" \o "Kwack, 2009 #14"]. A two-year dermal exposure study in rats and mice by [HYPERLINK \l "_ENREF_35" \o "NTP, 1995 #27"] provided information on tumor incidence. Other studies focused on general toxicity and hepatic effects, including organ weight, histopathology, and biochemical changes. This includes nine low dose (0.57 mg/kg-day to 2.85 mg/kg-day) studies in rats or mice performed by the same laboratory [ADDIN EN.CITE ADDIN EN.CITE.DATA], as well as the studies in rats by [HYPERLINK \l "_ENREF_32" \o "Moody, 1978 #16"] and [HYPERLINK \l "_ENREF_3" \o "Brown, 1978 #72"].

3.2. Study evaluation

Overall study confidence classifications by outcome are summarized in Table 1, and heat maps summarizing study evaluation ratings by domain are provided in the Supplementary Materials (Figure S1). Figure S1 provides links to an interactive figure in HAWC, where rationale for the study evaluation ratings is documented.

Based on the study evaluation considerations outlined in the systematic review protocol, confidence was reduced in some studies that had incomplete reporting of experimental designs or results. For example, dose-related histopathological findings were frequently reported qualitatively, with no quantitative data provided on the incidence or severity of lesions; such outcomes were considered *low confidence.* Additionally, some study outcomes were rated *medium* or *low confidence* due to sensitivity concerns with certain endpoint measurements. For instance, for evaluation of maternal body weight gain, rabbits were not considered an appropriate test species since body weight changes in rabbits are more variable compared to other species [HYPERLINK \l "_ENREF_63" \o "US EPA, 1991 #65"]; and, for all species, confidence was reduced in the maternal body weight gain measurements in studies that did not adjust for gravid uterine weight, which facilitates the interpretation of maternal toxicity relative to effects on fetal body weight [ADDIN EN.CITE <EndNote><Cite><Author>US EPA</Author><Year>1991</Year><RecNum>65</RecNum><DisplayText>(US EPA 1991)</DisplayText><record><rec-number>65</rec-number><foreign-keys><key app="EN" db-id="rpv9f5v5cfrzxgesv5bv9sdn0e9dz2vdpzwx" timestamp="0">65</key></foreign-keys><ref-type name="Government Document">46</ref-type><contributors><authors><author>US EPA,</author></authors><secondary-authors><author>Risk Assessment Forum</author></secondary-authors></contributors><titles><title>Guidelines for developmental toxicity risk assessment.</title></titles><dates><year>1991</year></dates><pub-location>Washington DC</pub-location><publisher>U.S. Environmental Protecion Agency</publisher><isbn>EPA/600/FR-91/001</isbn><urls></urls></record></Cite></EndNote>]. For organ weights, it was considered best practice for studies to report both absolute and relative (adjusted to body weight) measurements, although relative organ weight as a standalone measurement was generally considered to be acceptable for most organs [ADDIN EN.CITE <EndNote><Cite><Author>Bailey</Author><Year>2004</Year><RecNum>66</RecNum><DisplayText>(Bailey et al. 2004)</DisplayText><record><rec-number>66</rec-number><foreign-keys><key app="EN" db-id="rpv9f5v5cfrzxgesv5bv9sdn0e9dz2vdpzwx" timestamp="0">66</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Bailey, S. A.</author><author>Zidell, R. H.</author><author>Perry, R. W.</author></authors></contributors><auth-address>Wyeth Research, Chazy, New York 12921, USA. baileys@wyeth.com</auth-address><titles><title>Relationships between organ weight and body/brain weight in the rat: what is the best analytical endpoint?</title><secondary-title>Toxicol Pathol</secondary-title><alt-title>Toxicologic pathology</alt-title></titles><pages>448-66</pages><volume>32</volume><number>4</number><keywords><keyword>Animals</keyword><keyword>Body Weight/*drug effects</keyword><keyword>Brain/*drug

effects</keyword><keyword>Endpoint Determination/*veterinary</keyword><keyword>Female</keyword><keyword>Linear Models</keyword><keyword>Liver/*drug effects</keyword><keyword>Male</keyword><keyword>Organ Size/*drug effects</keyword><keyword>Organ Specificity/drug effects</keyword><keyword>Predictive Value of Tests</keyword><keyword>Rats</keyword><keyword>Rats, Inbred Strains</keyword><keyword>Retrospective Studies</keyword><keyword>Thyroid Gland/*drug effects</keyword><keyword>Toxicity Tests, Acute/*veterinary</keyword></keywords><dates><year>2004</year><pub-dates><date>Jul-Aug</date></pub-dates></dates><isbn>0192-6233 (Print)
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For testis, however, studies only reporting relative organ weight were considered *low confidence*, since testis weight is not proportionate to body weight [ADDIN EN.CITE <EndNote><Cite><Author>Bailey</Author><Year>2004</Year><RecNum>66</RecNum><DisplayText>(Bailey et al. 2004)</DisplayText><record><rec-number>66</rec-number><foreign-keys><key app="EN" db-id="rpv9f5v5cfrzxgesv5bv9sdn0e9dz2vdpzwx" timestamp="0">66</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Bailey, S. A.</author><author>Zidell, R. H.</author><author>Perry, R. W.</author></authors></contributors><auth-address>Wyeth Research, Chazy, New York 12921, USA. baileys@wyeth.com</auth-address><titles><title>Relationships between organ weight and body/brain weight in the rat: what is the best analytical endpoint?</title><secondary-title>Toxicol Pathol</secondary-title><alt-title>Toxicologic pathology</alt-title></titles><pages>448-66</pages><volume>32</volume><number>4</number><keywords><keyword>Animals</keyword><keyword>Body Weight/*drug effects</keyword><keyword>Brain/*drug effects</keyword><keyword>Endpoint Determination/*veterinary</keyword><keyword>Female</keyword><keyword>Linear Models</keyword><keyword>Liver/*drug effects</keyword><keyword>Male</keyword><keyword>Organ Size/*drug effects</keyword><keyword>Organ Specificity/drug effects</keyword><keyword>Predictive Value of Tests</keyword><keyword>Rats</keyword><keyword>Rats, Inbred Strains</keyword><keyword>Retrospective Studies</keyword><keyword>Thyroid Gland/*drug effects</keyword><keyword>Toxicity Tests, Acute/*veterinary</keyword></keywords><dates><year>2004</year><pub-dates><date>Jul-Aug</date></pub-dates></dates><isbn>0192-6233 (Print)
0192-6233 (Linking)</isbn><accession-num>15204968</accession-num><urls><related-urls><url>http://www.ncbi.nlm.nih.gov/pubmed/15204968</url></related-urls></urls><electronic-resource-num>10.1080/01926230490465874</electronic-resource-num></record></Cite></EndNote>].

Specific concerns were raised regarding studies from two laboratory groups that tested relatively low doses of DEP; these include concerns about exposure characterization as well as other concerns for risk of bias and sensitivity. The studies by [HYPERLINK \l "_ENREF_29" \o "Manservisi, 2015 #48"] and [HYPERLINK \l "_ENREF_21" \o "Hu, 2016 #47"], both of which reported data on the same group of animals, dosed rats with 0.173 mg/kg-day three times per week from birth through PND 181 and stated via personal correspondence that they verified the concentration of DEP in the dosing solutions but did not evaluate background levels of DEP that may be present due to the use of phthalate in plastics or other environmental sources. This is potentially problematic because a separate dose range-finding study by these authors [ADDIN EN.CITE <EndNote><Cite><Author>Teitelbaum</Author><Year>2016</Year><RecNum>75</RecNum><DisplayText>(Teitelbaum et al. 2016)</DisplayText><record><rec-number>75</rec-number><foreign-keys><key app="EN" db-id="rpv9f5v5cfrzxgesv5bv9sdn0e9dz2vdpzwx" timestamp="0">75</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Teitelbaum, S. L.</author><author>Li, Q.</author><author>Lambertini, L.</author><author>Belpoggi, F.</author><author>Manservisi, F.</author><author>Falcioni, L.</author><author>Bua, L.</author><author>Silva, M. J.</author><author>Ye, X.</author><author>Calafat, A. M.</author><author>Chen, J.</author></authors></contributors><titles><title>Paired serum and urine concentrations of biomarkers of diethyl phthalate, methyl paraben, and triclosan in rats</title><secondary-title>Environmental Health Perspectives</secondary-title><alt-title>Environ Health Perspect</alt-title></titles><pages>39-45</pages><volume>124</volume><number>1</number><dates><year>2016</year></dates><isbn>ISSN 0091-6765
EISSN 1552-9924</isbn><accession-num>26047088</accession-num><label>2918730</label><urls><related-urls><url>http://dx.doi.org/10.1289/ehp.1409586</url></related-urls></urls><electronic-resource-num>10.1289/ehp.1409586</electronic-resource-num><language>English</language></record></Cite></EndNote>] reported elevated levels of MEP in the urine of control animals, which suggests the potential for background exposures or contamination that could significantly impact the nominal dose levels. A series of studies by another laboratory exposed rats or mice to low doses of DEP in diet (0.57 mg/kg-day to 2.85 mg/kg-day) [ADDIN EN.CITE ADDIN EN.CITE.DATA] or to 50 ppm DEP in drinking water [ADDIN EN.CITE ADDIN EN.CITE.DATA] without verifying the nominal doses. Again, concerns were raised that undetected background levels of phthalates in control groups could mask true effects and reduce study sensitivity. Moreover, several of these studies by Pereira and coauthors stated that the concentration of DEP in the diet was increased on weekly basis to maintain the dose in proportion with the animals' body weight but did not provide additional information on this dose adjustment, which raises separate questions about the accuracy of the nominal doses in these studies. Studies by these two laboratory groups also had other concerns raised during study evaluation; for instance, data from littermates were presented as the average of individual pups, which has the potential to overestimate the statistical significance of experimental findings [ADDIN EN.CITE <EndNote><Cite><Author>Haseman</Author><Year>2001</Year><RecNum>70</RecNum><DisplayText>(Haseman et al. 2001)</DisplayText><record><rec-number>70</rec-number><foreign-keys><key app="EN" db-id="rpv9f5v5cfrzxgesv5bv9sdn0e9dz2vdpzwx" timestamp="0">70</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Haseman, J. K.</author><author>Bailer, A. J.</author><author>Kodell, R. L.</author><author>Morris, R.</author><author>Portier, K.</author></authors></contributors><titles><title>Statistical issues in the analysis of low-dose endocrine disruptor data</title><secondary-title>Toxicological Sciences</secondary-title><alt-title>Toxicol Sci</alt-title></titles><periodical><full-title>Toxicological Sciences</full-title><abbr-1>Toxicol Sci</abbr-1></periodical><alt-periodical><full-title>Toxicological Sciences</full-title><abbr-1>Toxicol Sci</abbr-1></alt-periodical><pages>201-

210</pages><volume>61</volume><number>2</number><dates><year>2001</year></dates><isbn>ISSN 1096-6080
EISSN 1096-0929</isbn><accession-num>11353128</accession-num><label>192698</label><urls></urls><language>English</language></record></Cite></EndNote>]. Most of these studies also described histopathological changes in the exposed animals without providing quantitative data to support their findings. Overall, due to these cumulative concerns, these studies were rated as *low confidence* for all reported outcomes.

3.3. Male reproductive effects

Male reproductive effects were evaluated according to the life stage of exposure [ADDIN EN.CITE <EndNote><Cite><Author>US EPA</Author><Year>2006</Year><RecNum>64</RecNum><DisplayText>(US EPA 2006)</DisplayText><record><rec-number>64</rec-number><foreign-keys><key app="EN" db-id="rpv9f5v5cfrzxgesv5bv9sdn0e9dz2vdpzwx" timestamp="0">64</key></foreign-keys><ref-type name="Government Document">46</ref-type><contributors><authors><author>US EPA,</author></authors><secondary-authors><author>Office of Research and Development, National Center for Environmental Assessment</author></secondary-authors></contributors><titles><title>A Framework for assessing health risk of environmental exposures to children</title></titles><dates><year>2006</year></dates><pub-location>Washington, DC</pub-location><isbn>EPA/600/R-05/093F</isbn><urls></urls><custom1>Environmental Protection Agency</custom1></record></Cite></EndNote>]. Figures indicating the doses at which statistically significant male reproductive effects occurred are provided in Supplemental Materials (Figures S2 – S4).

3.3.1. Summary of gestational and early postnatal exposure studies (including F1 or F2 offspring from multigenerational studies)

Three *high* or *medium confidence* studies investigated effects on testosterone production or levels in male rats following gestational exposure [ADDIN EN.CITE ADDIN EN.CITE.DATA] or exposure from gestation through PND 3 [ADDIN EN.CITE <EndNote><Cite><Author>Gray</Author><Year>2000</Year><RecNum>10</RecNum><DisplayText>(Gray et al. 2000)</DisplayText><record><rec-number>10</rec-number><foreign-keys><key app="EN" db-id="rpv9f5v5cfrzxgesv5bv9sdn0e9dz2vdpzwx" timestamp="0">10</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Gray, L. E., Jr</author><author>Ostby, J.</author><author>Furr, J.</author><author>Price, M.</author><author>Veeramachaneni, D. N. R.</author><author>Parks, L.</author></authors></contributors><titles><title>Perinatal exposure to the phthalates DEHP, BBP, and DNIP, but not DEP, DMP, or DOTP, alters sexual differentiation of the male rat</title><secondary-title>Toxicological Sciences</secondary-title><alt-title>Toxicol Sci</alt-title><short-title>Toxicological Sciences</short-title></titles><periodical><full-title>Toxicological Sciences</full-title><abbr-1>Toxicol Sci</abbr-1></periodical><alt-periodical><full-title>Toxicological Sciences</full-title><abbr-1>Toxicol Sci</abbr-1></alt-periodical><pages>350-365</pages><volume>58</volume><number>2</number><dates><year>2000</year></dates><isbn>ISSN 1096-6080
EISSN 1096-0929</isbn><accession-num>11099647</accession-num><label>678742</label><urls><related-urls><url>https://heronet.epa.gov/heronet/index.cfm/reference/download/reference_id/678742C3 -1097,1547,1826,2205,2206,2207,2212,2245,2247,2294,2305,2366,2370,2424,2516,2642</url></related-urls></urls><custom1>true</custom1><electronic-resource-num>10.1093/toxsci/58.2.350</electronic-

resource-num><language>English</language></record></Cite></EndNote>]. These studies all used
exposure periods that included the critical window of male sexual differentiation in late gestation (which
occurs between ~GD 14-18), and therefore are considered relevant for the evaluation of fetal
testosterone production and other phthalate syndrome effects. No significant effects on fetal testicular
testosterone production were observed at GD 18 following exposure from GD 14-18 [ADDIN EN.CITE
<EndNote><Cite><Author>Furr</Author><Year>2014</Year><RecNum>42</RecNum><DisplayText>(Fur
r et al. 2014)</DisplayText><record><rec-number>42</rec-number><foreign-keys><key app="EN" db-
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R.</author><author>Lambright, C. S.</author><author>Wilson, V. S.</author><author>Foster, P.
M.</author><author>Gray, L. E., Jr</author></authors></contributors><titles><title>A short-term in
vivo screen using fetal testosterone production, a key event in the phthalate adverse outcome pathway,
to predict disruption of sexual differentiation</title><secondary-title>Toxicological
Sciences</secondary-title><alt-title>Toxicol Sci</alt-title><short-title>Toxicological Sciences</short-
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resource-num><language>English</language></record></Cite></EndNote>] or GD 8-18 [ADDIN EN.CITE
<EndNote><Cite><Author>Howdeshell</Author><Year>2008</Year><RecNum>8</RecNum><DisplayTe
xt>(Howdeshell et al. 2008)</DisplayText><record><rec-number>8</rec-number><foreign-keys><key
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L.</author><author>Wilson, V. S.</author><author>Furr, J.</author><author>Lambright, C.
R.</author><author>Rider, C. V.</author><author>Blystone, C. R.</author><author>Hotchkiss, A.
K.</author><author>Gray, L. E., Jr</author></authors></contributors><titles><title>A mixture of five
phthalate esters inhibits fetal testicular testosterone production in the Sprague-Dawley rat in a
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165</pages><volume>105</volume><number>1</number><dates><year>2008</year></dates><isbn>I
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6</url></related-urls></urls><custom1>true</custom1><electronic-resource-
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num><language>English</language></record></Cite></EndNote>] at doses up to 750 and 900 mg/kg-

day, respectively. Likewise, there was no significant effect on serum testosterone levels in adult males that had been exposed to 750 mg/kg-day DEP from GD 14 – PND 3 [ADDIN EN.CITE <EndNote><Cite><Author>Gray</Author><Year>2000</Year><RecNum>10</RecNum><DisplayText>(Gray et al. 2000)</DisplayText><record><rec-number>10</rec-number><foreign-keys><key app="EN" db-id="rpv9f5v5cfrzxgesv5bv9sdn0e9dz2vdpzwx" timestamp="0">10</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Gray, L. E., Jr</author><author>Ostby, J.</author><author>Furr, J.</author><author>Price, M.</author><author>Veeramachaneni, D. N. R.</author><author>Parks, L.</author></authors></contributors><titles><title>Perinatal exposure to the phthalates DEHP, BBP, and DNIP, but not DEP, DMP, or DOTP, alters sexual differentiation of the male rat</title><secondary-title>Toxicological Sciences</secondary-title><alt-title>Toxicol Sci</alt-title><short-title>Toxicological Sciences</short-title></titles><periodical><full-title>Toxicological Sciences</full-title><abbr-1>Toxicol Sci</abbr-1></periodical><alt-periodical><full-title>Toxicological Sciences</full-title><abbr-1>Toxicol Sci</abbr-1></alt-periodical><pages>350-365</pages><volume>58</volume><number>2</number><dates><year>2000</year></dates><isbn>ISSN 1096-6080
EISSN 1096-0929</isbn><accession-num>11099647</accession-num><label>678742</label><urls><related-urls><url>https://heronet.epa.gov/heronet/index.cfm/reference/download/reference_id/678742C3 - 1097,1547,1826,2205,2206,2207,2212,2245,2247,2294,2305,2366,2370,2424,2516,2642</url></related-urls></urls><custom1>true</custom1><electronic-resource-num>10.1093/toxsci/58.2.350</electronic-resource-num><language>English</language></record></Cite></EndNote>]. Additional supporting mechanistic evidence was provided by [HYPERLINK \l "_ENREF_26" \o "Liu, 2005 #80"], who did not report testosterone production but evaluated global gene expression in the fetal rat testis after exposure to 500 mg/kg-day from GD 12-19; gene expression in DEP-treated animals was similar to controls, whereas known antiandrogenic phthalates (e.g. dibutyl phthalate, diethylhexyl phthalate) downregulated expression of genes involved in cholesterol homeostasis and steroidogenesis [ADDIN EN.CITE <EndNote><Cite><Author>Liu</Author><Year>2005</Year><RecNum>80</RecNum><DisplayText>(Liu et al. 2005)</DisplayText><record><rec-number>80</rec-number><foreign-keys><key app="EN" db-id="rpv9f5v5cfrzxgesv5bv9sdn0e9dz2vdpzwx" timestamp="1544705029">80</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Liu, K.</author><author>Lehmann, K. P.</author><author>Sar, M.</author><author>Young, S. S.</author><author>Gaido, K. W.</author></authors></contributors><titles><title>Gene expression profiling following in utero exposure to phthalate esters reveals new gene targets in the etiology of testicular dysgenesis</title><secondary-title>Biology of Reproduction</secondary-title><alt-title>Biol Reprod</alt-title></titles><periodical><full-title>Biology of Reproduction</full-title><abbr-1>Biol Reprod</abbr-1></periodical><alt-periodical><full-title>Biology of Reproduction</full-title><abbr-1>Biol Reprod</abbr-1></alt-periodical><pages>180-192</pages><volume>73</volume><number>1</number><dates><year>2005</year></dates><isbn>ISSN 0006-3363
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"_ENREF_4" \o "Clewell, 2010 #7"] evaluated testicular MEP levels in a subset of male fetuses from the study by [HYPERLINK \l "_ENREF_26" \o "Liu, 2005 #80"] and found MEP present at relatively high levels, indicating that the lack of effect of DEP in the study by [HYPERLINK \l "_ENREF_26" \o "Liu, 2005 #80"] was not due to the dose not reaching the testis. Taken together, these studies consistently suggest lack of effect on fetal testosterone production; however, additional evidence (e.g. studies in other species besides rat) would be needed to conclude that there was compelling evidence of no effect. The evidence for effects on testosterone levels after gestational exposure was therefore considered *indeterminate*.

Male reproductive organ weights and other biomarkers of androgen-dependent reproductive development were evaluated in F1 peripubertal and adult rats after exposure from GD 14 – PND 3 [ADDIN EN.CITE
<EndNote><Cite><Author>Gray</Author><Year>2000</Year><RecNum>10</RecNum><DisplayText>(Gr
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M.</author><author>Veeramachaneni, D. N. R.</author><author>Parks,
L.</author></authors></contributors><titles><title>Perinatal exposure to the phthalates DEHP, BBP,
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EISSN 1096-0929</isbn><accession-num>11099647</accession-
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1097,1547,1826,2205,2206,2207,2212,2245,2247,2294,2305,2366,2370,2424,2516,2642</url></related
-urls></urls><custom1>true</custom1><electronic-resource-num>10.1093/toxsci/58.2.350</electronic-
resource-num><language>English</language></record></Cite></EndNote>] and in F1 and F2 weanling rats in the two-generation reproduction study [ADDIN EN.CITE
<EndNote><Cite><Author>Fujii</Author><Year>2005</Year><RecNum>22</RecNum><DisplayText>(Fuj
ii et al. 2005)</DisplayText><record><rec-number>22</rec-number><foreign-keys><key app="EN" db-
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name="Journal Article">17</ref-type><contributors><authors><author>Fujii, S.</author><author>Yabe,
K.</author><author>Furukawa, M.</author><author>Hirata, M.</author><author>Kiguchi,
M.</author><author>Ikka, T.</author></authors></contributors><titles><title>A two-generation
reproductive toxicity study of diethyl phthalate (DEP) in rats</title><secondary-title>Journal of
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116</pages><volume>30</volume><number>Special Issue
P</number><dates><year>2005</year></dates><isbn>ISSN 0388-1350
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1097,2187,2212,2245,2294,2305,2344</url></related-urls></urls><custom1>true</custom1><electronic-resource-num>10.2131/jts.30.S97</electronic-resource-num><language>English</language></record></Cite></EndNote>]. Additionally, AGD was evaluated in fetal rats after exposure from GD 12-19 [ADDIN EN.CITE <EndNote><Cite><Author>Liu</Author><Year>2005</Year><RecNum>80</RecNum><DisplayText>(Liu et al. 2005)</DisplayText><record><rec-number>80</rec-number><foreign-keys><key app="EN" db-id="rpv9f5v5cfrzxgesv5bv9sdn0e9dz2vdpzwx" timestamp="1544705029">80</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Liu, K.</author><author>Lehmann, K. P.</author><author>Sar, M.</author><author>Young, S. S.</author><author>Gaido, K. W.</author></authors></contributors><titles><title>Gene expression profiling following in utero exposure to phthalate esters reveals new gene targets in the etiology of testicular dysgenesis</title><secondary-title>Biology of Reproduction</secondary-title><alt-title>Biol Reprod</alt-title></titles><periodical><full-title>Biology of Reproduction</full-title><abbr-1>Biol Reprod</abbr-1></periodical><alt-periodical><full-title>Biology of Reproduction</full-title><abbr-1>Biol Reprod</abbr-1></alt-periodical><pages>180-192</pages><volume>73</volume><number>1</number><dates><year>2005</year></dates><isbn>ISSN 0006-3363
EISSN 1529-7268</isbn><accession-num>15728792</accession-num><label>674387</label><urls><related-urls><url>http://dx.doi.org/10.1095/biolreprod.104.039404</url></related-urls></urls><electronic-resource-num>10.1095/biolreprod.104.039404</electronic-resource-num><language>English</language></record></Cite></EndNote>]. All of these studies were judged to be *high confidence* for these outcomes. There were no statistically significant effects on male reproductive organ weights [ADDIN EN.CITE ADDIN EN.CITE.DATA], with the exception of a 20% decrease in absolute prostate weights and 17% increase in relative seminal vesical weight observed in F1 weanlings exposed to 1016 mg/kg-day DEP [ADDIN EN.CITE <EndNote><Cite><Author>Fujii</Author><Year>2005</Year><RecNum>22</RecNum><DisplayText>(Fujii et al. 2005)</DisplayText><record><rec-number>22</rec-number><foreign-keys><key app="EN" db-id="rpv9f5v5cfrzxgesv5bv9sdn0e9dz2vdpzwx" timestamp="0">22</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Fujii, S.</author><author>Yabe, K.</author><author>Furukawa, M.</author><author>Hirata, M.</author><author>Kiguchi, M.</author><author>Ikka, T.</author></authors></contributors><titles><title>A two-generation reproductive toxicity study of diethyl phthalate (DEP) in rats</title><secondary-title>Journal of Toxicological Sciences</secondary-title><alt-title>J Toxicol Sci</alt-title><short-title>Journal of Toxicological Sciences</short-title></titles><pages>S97-116</pages><volume>30</volume><number>Special Issue P</number><dates><year>2005</year></dates><isbn>ISSN 0388-1350
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id="rpv9f5v5cfrzxgesv5bv9sdn0e9dz2vdpzwx" timestamp="0">22</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Fujii, S.</author><author>Yabe, K.</author><author>Furukawa, M.</author><author>Hirata, M.</author><author>Kiguchi, M.</author><author>Ikka, T.</author></authors></contributors><titles><title>A two-generation reproductive toxicity study of diethyl phthalate (DEP) in rats</title><secondary-title>Journal of Toxicological Sciences</secondary-title><alt-title>J Toxicol Sci</alt-title><short-title>Journal of Toxicological Sciences</short-title></titles><pages>S97-116</pages><volume>30</volume><number>Special Issue P</number><dates><year>2005</year></dates><isbn>ISSN 0388-1350
EISSN 1880-3989</isbn><accession-num>16641546</accession-num><label>1298274</label><urls><related-urls><url>https://heronet.epa.gov/heronet/index.cfm/reference/download/reference_id/1298274C3 - 1097,2187,2212,2245,2294,2305,2344</url></related-urls></urls><custom1>true</custom1><electronic-resource-num>10.2131/jts.30.S97</electronic-resource-num><language>English</language></record></Cite></EndNote>]. There were no effects on AGD [ADDIN EN.CITE ADDIN EN.CITE.DATA] or nipple retention [ADDIN EN.CITE <EndNote><Cite><Author>Gray</Author><Year>2000</Year><RecNum>10</RecNum><DisplayText>(Gray et al. 2000)</DisplayText><record><rec-number>10</rec-number><foreign-keys><key app="EN" db-id="rpv9f5v5cfrzxgesv5bv9sdn0e9dz2vdpzwx" timestamp="0">10</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Gray, L. E., Jr</author><author>Ostby, J.</author><author>Furr, J.</author><author>Price, M.</author><author>Veeramachaneni, D. N. R.</author><author>Parks, L.</author></authors></contributors><titles><title>Perinatal exposure to the phthalates DEHP, BBP, and DNIP, but not DEP, DMP, or DOTP, alters sexual differentiation of the male rat</title><secondary-title>Toxicological Sciences</secondary-title><alt-title>Toxicol Sci</alt-title><short-title>Toxicological Sciences</short-title></titles><periodical><full-title>Toxicological Sciences</full-title><abbr-1>Toxicol Sci</abbr-1></periodical><alt-periodical><full-title>Toxicological Sciences</full-title><abbr-1>Toxicol Sci</abbr-1></alt-periodical><pages>350-365</pages><volume>58</volume><number>2</number><dates><year>2000</year></dates><isbn>ISSN 1096-6080
EISSN 1096-0929</isbn><accession-num>11099647</accession-num><label>678742</label><urls><related-urls><url>https://heronet.epa.gov/heronet/index.cfm/reference/download/reference_id/678742C3 - 1097,1547,1826,2205,2206,2207,2212,2245,2247,2294,2305,2366,2370,2424,2516,2642</url></related-urls></urls><custom1>true</custom1><electronic-resource-num>10.1093/toxsci/58.2.350</electronic-resource-num><language>English</language></record></Cite></EndNote>], both of which are biomarkers of androgen-dependent reproductive development. Gestational exposure to DEP did not affect the timing of sexual maturation, as measured by the age at onset of preputial separation [ADDIN EN.CITE ADDIN EN.CITE.DATA]. The lack of effect on these outcomes is consistent with the observation that DEP does not affect testosterone in rats exposed during gestation. However, since a larger body of evidence would be needed to conclude there was compelling evidence of no effect, the evidence for effects on male reproductive organ weights and morphological development were both considered *indeterminate.*

3.3.2. Summary of peripubertal and adult exposure studies (including F0 or F1 parental animals from multigenerational studies)

In contrast with results observed after gestational exposure, several studies reported decreased testosterone levels following peripubertal or adult exposure to DEP in male rats. In a *high confidence* two-generation reproduction study, [HYPERLINK \l "_ENREF_10" \o "Fujii, 2005 #22"] observed decreased serum testosterone in F0 males following 14 weeks of exposure in all dose groups, reaching statistical significance in the two highest dose groups (197 and 1016 mg/kg-day); however, this evaluation was performed on a relatively small subset of the animals (n= 6/group) and results showed a large amount of variability with a nonmonotonic magnitude of change (decreased by 80% in the 197 mg/kg-day group and 50% in the 1016 mg/kg-day group). This study did not evaluate hormone levels in F1 animals, so it is not clear whether this effect was present across other generations; however, it is notable that there was minimal or no effect in these animals on male reproductive organ weights (discussed below), which are known to be sensitive to changes in androgen levels [ADDIN EN.CITE <EndNote><Cite><Author>US EPA</Author><Year>1996</Year><RecNum>92</RecNum><DisplayText>(US EPA 1996)</DisplayText><record><rec-number>92</rec-number><foreign-keys><key app="EN" db-id="rpv9f5v5cfrzxgesv5bv9sdn0e9dz2vdpzwx" timestamp="1560871846">92</key></foreign-keys><ref-type name="Government Document">46</ref-type><contributors><authors><author>US EPA,</author></authors><tertiary-authors><author>U.S. Environmental Protection Agency, Risk Assessment Forum</author></tertiary-authors></contributors><titles><title>Guidelines for reproductive toxicity risk assessment</title></titles><pages>1-143</pages><dates><year>1996</year></dates><pub-location>Washington ,DC</pub-location><publisher>U.S. Environmental Protection Agency</publisher><isbn>EPA/630/R-96/009</isbn><urls></urls><electronic-resource-num>https://www.epa.gov/sites/production/files/2014-11/documents/guidelines_repro_toxicity.pdf</electronic-resource-num></record></Cite></EndNote>]. Dose-related statistically significant decreases in serum testosterone and androstenedione were also observed in adult male rats exposed to 0.57 mg/kg-day to 2.85 mg/kg-day DEP for 150 days in the study by [HYPERLINK \l "_ENREF_46" \o "Pereira, 2008 #34"], which is considered *low confidence* for this outcome due to the study design concerns described in Section 3.2. In addition, serum and testicular testosterone levels were statistically significantly decreased in young rats exposed to DEP for 7 days [ADDIN EN.CITE <EndNote><Cite><Author>Oishi</Author><Year>1980</Year><RecNum>2</RecNum><DisplayText>(Oishi and Hiraga 1980)</DisplayText><record><rec-number>2</rec-number><foreign-keys><key app="EN" db-id="rpv9f5v5cfrzxgesv5bv9sdn0e9dz2vdpzwx" timestamp="0">2</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Oishi, S.</author><author>Hiraga, K.</author></authors></contributors><titles><title>Testicular atrophy induced by phthalic acid esters: Effect on testosterone and zinc concentrations</title><secondary-title>Toxicology and Applied Pharmacology</secondary-title><alt-title>Toxicol Appl Pharmacol</alt-title><short-title>Toxicology and Applied Pharmacology</short-title></titles><pages>35-41</pages><volume>53</volume><number>1</number><dates><year>1980</year></dates><isbn>ISSN 0041-008X
EISSN 1096-0333</isbn><accession-num>7385236</accession-num><label>61572</label><urls><related-urls><url>https://heronet.epa.gov/heronet/index.cfm/reference/download/reference_id/61572C3 -14,31,32,1097,1480,1547,1628,1826,1837,2188,2195,2205,2206,2265,2294,2305,2320</url></related-urls></urls><custom1>true</custom1><electronic-resource-num>10.1016/0041-008x(80)90378-

6</electronic-resource-num><language>English</language></record></Cite></EndNote>], although this data was presented as a percentage of control without a measure of variance and is considered *low confidence*. In contrast to other findings, no effect on serum testosterone or gonadotropin levels was observed in a *high confidence* study in Wistar rats following exposures up to 1000 mg/kg-day DEP for 28 days [ADDIN EN.CITE <EndNote><Cite><Author>Shiraishi</Author><Year>2006</Year><RecNum>30</RecNum><DisplayText>(Shiraishi et al. 2006)</DisplayText><record><rec-number>30</rec-number><foreign-keys><key app="EN" db-id="rpv9f5v5cfrzxgesv5bv9sdn0e9dz2vdpzwx" timestamp="0">30</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Shiraishi, K.</author><author>Miyata, K.</author><author>Houshuyama, S.</author><author>Imatanaka, N.</author><author>Umano, T.</author><author>Minobe, Y.</author><author>Yamasaki, K.</author></authors></contributors><titles><title>Subacute oral toxicity study of diethylphthalate based on the draft protocol for "Enhanced OECD Test Guideline no. 407&quot</title><secondary-title>Archives of Toxicology</secondary-title><alt-title>Arch Toxicol</alt-title><short-title>Archives of Toxicology</short-title></titles><pages>10-16</pages><volume>80</volume><number>1</number><dates><year>2006</year></dates><isbn>ISSN 0340-5761
EISSN 1432-0738</isbn><accession-num>16059724</accession-num><label>1315363</label><urls><related-urls><url>https://heronet.epa.gov/heronet/index.cfm/reference/download/reference_id/1315363C3 -1097,2204,2212,2305,2516</url></related-urls></urls><custom1>true</custom1><electronic-resource-num>10.1007/s00204-005-0008-6</electronic-resource-num><language>English</language></record></Cite></EndNote>], although those authors did observe a significant decrease in serum estradiol for males in the 1000 mg/kg-day dose group. Additional supporting mechanistic evidence was available in the study in rats by [HYPERLINK \l "_ENREF_9" \o "Foster, 1983 #86"], which found that peripubertal exposure to ~1600 mg/kg-day DEP for up to 4 days did not affect the activity of testicular steroidogenic enzymes, whereas exposure to a known antiandrogenic phthalate (dipentyl phthalate) decreased the enzyme activity in a time-dependent manner. Overall, although decreased testosterone was observed in three studies, two of these studies had significant concerns for bias and the results are not supported by mechanistic evidence or other coherent effects (e.g. effects on testis weight) in the *high confidence* study by [HYPERLINK \l "_ENREF_10" \o "Fujii, 2005 #22"]. The evidence for effects on testosterone after peripubertal or adult exposure was considered *slight*.

Three *high confidence* studies that evaluated sperm parameters in rats or mice found statistically significant effects on sperm count, motility, or morphology following multigenerational exposure to DEP [ADDIN EN.CITE ADDIN EN.CITE.DATA] or peripubertal exposure to DEP or MEP [ADDIN EN.CITE <EndNote><Cite><Author>Kwack</Author><Year>2009</Year><RecNum>14</RecNum><DisplayText>(Kwack et al. 2009)</DisplayText><record><rec-number>14</rec-number><foreign-keys><key app="EN" db-id="rpv9f5v5cfrzxgesv5bv9sdn0e9dz2vdpzwx" timestamp="0">14</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Kwack, S.</author><author>Kim, K.</author><author>Kim, H.</author><author>Lee, B.</author></authors></contributors><titles><title>Comparative toxicological evaluation of phthalate diesters and metabolites in Sprague-Dawley male rats for risk assessment</title><secondary-title>Journal of Toxicology and Environmental Health, Part A: Current Issues</secondary-title><alt-title>J Toxicol Environ Health A</alt-title><short-title>Journal of Toxicology and Environmental Health,

Part A: Current Issues</short-title></titles><pages>1446-1454</pages><volume>72</volume><number>21-22</number><dates><year>2009</year></dates><isbn>ISSN 1528-7394
EISSN 1087-2620</isbn><accession-num>20077217</accession-num><label>697382</label><urls><related-urls><url>https://heronet.epa.gov/heronet/index.cfm/reference/download/reference_id/697382C3 - 1097,1480,1547,1826,1837,2008,2188,2205,2206,2207,2212,2247,2265,2294,2305</url></related-urls></urls><custom1>true</custom1><electronic-resource-num>10.1080/15287390903212923</electronic-resource-num><language>English</language></record></Cite></EndNote>]. [HYPERLINK \l "_ENREF_10" \o "Fujii, 2005 #22"] observed a statistically significant increase in abnormal or tailless sperm in both F0 and F1 rats, although the magnitude of effect was low (abnormal sperm rate was 1.52% in F1 animals in the 1150 mg/kg-day group versus 0.6% in control animals) and effects in F0 animals were nonmonotonic (occurring at 197 mg/kg-day group but not 1016 mg/kg-day). Sperm counts and motility were not affected in this study. [HYPERLINK \l "_ENREF_23" \o "Kwack, 2009 #14"] observed that epididymal sperm count and percent motility were statistically significantly decreased in rats following exposure to 250 mg/kg-day MEP for four weeks, while percent linearity was statistically significantly decreased at 500 mg/kg-day DEP, with no effects on other sperm parameters. [HYPERLINK \l "_ENREF_54" \o "RTI International, 1984 #28"] reported that epididymal sperm counts were statistically significantly decreased in F1 mice dosed with 3640 mg/kg-day, with no effects on percent motility, abnormal sperm, or tailless sperm. Conversely, [HYPERLINK \l "_ENREF_57" \o "Shiraishi, 2006 #30"] reported no effects on epididymal sperm counts or morphology in adult male rats following 4-week DEP exposure at doses up to 1000 mg/kg-day, although the authors provided no quantitative data and this result is considered *low confidence*. Although there was variation in the effects observed across studies, the three available *high confidence* studies all observed some alterations in sperm quality, and therefore the evidence for effects on sperm parameters is considered *moderate.*

No effects on copulation or fertility in F0 or F1 mating pairs were observed in two multigenerational studies that are considered *high confidence* for this endpoint [ADDIN EN.CITE ADDIN EN.CITE.DATA]. Since only two studies were available, the evidence for effects on male fertility is considered *indeterminate*.

Reproductive organ weights in males following adult exposure to DEP were measured in several studies. In *high confidence* studies that reported absolute organ weights, DEP generally did not affect testosterone-dependent male reproductive organ weights (e.g., testes, epididymides, prostate, seminal vesicles) in F0 and F1 parental rats and mice [ADDIN EN.CITE ADDIN EN.CITE.DATA], although [HYPERLINK \l "_ENREF_10" \o "Fujii, 2005 #22"] reported a slight (5%) but statistically significant decrease in epididymal weights in F0 males at 1016 mg/kg-day. In *low confidence* studies, effects were inconsistent. The multigenerational study in rats by [HYPERLINK \l "_ENREF_41" \o "Pereira, 2007 #38"] reported statistically significantly decreased absolute testis weights in F0 parental males but not adult F1 males exposed to 2.85 mg/kg-day in diet, and [HYPERLINK \l "_ENREF_46" \o "Pereira, 2008 #34"] reported a statistically significant dose-related decrease in absolute testis and epididymis weights in adult male rats after exposure to 0.57 to 2.85 mg/kg-day. [HYPERLINK \l "_ENREF_3" \o "Brown, 1978 #72"] reported a statistically significant increase in relative testis weights in rats after 2, 6, or 16 weeks of exposure to 3160 mg/kg-day; however, it is possible that this is an artifact of decreased body weight in these animals, since testis and body weights are not proportional [ADDIN EN.CITE

<EndNote><Cite><Author>Bailey</Author><Year>2004</Year><RecNum>66</RecNum><DisplayText>(Bailey et al. 2004)</DisplayText><record><rec-number>66</rec-number><foreign-keys><key app="EN" db-id="rpv9f5v5cfrzxgesv5bv9sdn0e9dz2vdpzwx" timestamp="0">66</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Bailey, S. A.</author><author>Zidell, R. H.</author><author>Perry, R. W.</author></authors></contributors><auth-address>Wyeth Research, Chazy, New York 12921, USA. baileys@wyeth.com</auth-address><titles><title>Relationships between organ weight and body/brain weight in the rat: what is the best analytical endpoint?</title><secondary-title>Toxicol Pathol</secondary-title><alt-title>Toxicologic pathology</alt-title></titles><pages>448-66</pages><volume>32</volume><number>4</number><keywords><keyword>Animals</keyword><keyword>Body Weight/*drug effects</keyword><keyword>Brain/*drug effects</keyword><keyword>Endpoint Determination/*veterinary</keyword><keyword>Female</keyword><keyword>Linear Models</keyword><keyword>Liver/*drug effects</keyword><keyword>Male</keyword><keyword>Organ Size/*drug effects</keyword><keyword>Organ Specificity/drug effects</keyword><keyword>Predictive Value of Tests</keyword><keyword>Rats</keyword><keyword>Rats, Inbred Strains</keyword><keyword>Retrospective Studies</keyword><keyword>Thyroid Gland/*drug effects</keyword><keyword>Toxicity Tests, Acute/*veterinary</keyword></keywords><dates><year>2004</year><pub-dates><date>Jul-Aug</date></pub-dates></dates><isbn>0192-6233 (Print)
0192-6233 (Linking)</isbn><accession-num>15204968</accession-num><urls><related-urls><url>http://www.ncbi.nlm.nih.gov/pubmed/15204968</url></related-urls></urls><electronic-resource-num>10.1080/01926230490465874</electronic-resource-num></record></Cite></EndNote>]. In other studies that did not observe significant effects on body weight, relative testis weights were not affected by DEP [ADDIN EN.CITE ADDIN EN.CITE.DATA]. Given that absolute organ weights were largely unaffected in *high* confidence studies in rats and mice, the evidence for effects on male reproductive organ weights is considered *indeterminate*.

In addition, three studies [ADDIN EN.CITE ADDIN EN.CITE.DATA] performed general histopathological evaluations of male reproductive organs and did not observe any treatment-related findings, although none of these studies provided quantitative data to support their observations. Given this limited dataset, the evidence for histopathological effects in male reproductive organs is considered *indeterminant*.

3.3.3. Synthesis of results for male reproductive effects

Overall, the available studies suggest that there is *moderate* evidence that DEP is a male reproductive toxicant (Table 2). Decreased sperm quantity or quality was observed across three *high confidence* studies, although the magnitude of effect was low in some cases and there was variation in the parameters affected across studies. The observed effects on sperm are nevertheless compelling because similar effects have been reported for other phthalates, and are thought to result from direct targeting of Sertoli cells, seminiferous cords, and germ cells that occurs via an androgen-independent mode of action [ADDIN EN.CITE ADDIN EN.CITE.DATA]; it is plausible that DEP may operate through a similar mechanism, leading to effects on sperm in absence of an effect on steroidogenesis. Decreased testosterone was also observed in three peripubertal or adult exposure studies, although there is less confidence in that finding. Gestational exposure studies did not show effects of DEP on testosterone production, biomarkers of male reproductive development, sexual maturation, or (to any great extent) male reproductive organ weights.

Despite the effects on sperm parameters, there is no evidence that fertility was affected in the two-generation study by [HYPERLINK \l "_ENREF_10" \o "Fujii, 2005 #22"] or the continuous breeding study by [HYPERLINK \l "_ENREF_54" \o "RTI International, 1984 #28"]. However, it has been demonstrated that rodents can remain fertile even after dramatic reductions in sperm counts, whereas a relatively small change in sperm count may impact human fertility [ADDIN EN.CITE ADDIN EN.CITE.DATA]. Therefore, the findings of decreased sperm quality in animal models is of potential relevance for human health risk assessment.

Strengths of this evidence base include the availability of several *high confidence* gestational exposure studies, with two large multigenerational studies that assessed multiple outcomes in postnatal and adult animals [ADDIN EN.CITE ADDIN EN.CITE.DATA]. Other strengths are the availability of studies in both rats and mice that assessed similar outcomes, although the majority of evidence was from studies in rats.

3.4. Female reproductive effects

Figures indicating the doses at which statistically significant female reproductive effects occurred are provided in the Supplementary Materials (Figures S5 – S7 and S18)

3.4.1. Summary of available studies

Effects on pregnancy outcomes (including mating, fertility, fecundity, and gestation length) were evaluated in two *high confidence* studies following continuous DEP exposure across multiple generations in rats [ADDIN EN.CITE
<EndNote><Cite><Author>Fujii</Author><Year>2005</Year><RecNum>22</RecNum><DisplayText>(Fujii et al. 2005)</DisplayText><record><rec-number>22</rec-number><foreign-keys><key app="EN" db-id="rpv9f5v5cfrzxgesv5bv9sdn0e9dz2vdpzwx" timestamp="0">22</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Fujii, S.</author><author>Yabe, K.</author><author>Furukawa, M.</author><author>Hirata, M.</author><author>Kiguchi, M.</author><author>Ikka, T.</author></authors></contributors><titles><title>A two-generation reproductive toxicity study of diethyl phthalate (DEP) in rats</title><secondary-title>Journal of Toxicological Sciences</secondary-title><alt-title>J Toxicol Sci</alt-title><short-title>Journal of Toxicological Sciences</short-title></titles><pages>S97-116</pages><volume>30</volume><number>Special Issue P</number><dates><year>2005</year></dates><isbn>ISSN 0388-1350
EISSN 1880-3989</isbn><accession-num>16641546</accession-num><label>1298274</label><urls><related-urls><url>https://heronet.epa.gov/heronet/index.cfm/reference/download/reference_id/1298274C3 -1097,2187,2212,2245,2294,2305,2344</url></related-urls></urls><custom1>true</custom1><electronic-resource-num>10.2131/jts.30.S97</electronic-resource-num><language>English</language></record></Cite></EndNote>] and mice [ADDIN EN.CITE
<EndNote><Cite><Author>RTI International</Author><Year>1984</Year><RecNum>28</RecNum><DisplayText>(RTI International 1984)</DisplayText><record><rec-number>28</rec-number><foreign-keys><key app="EN" db-id="rpv9f5v5cfrzxgesv5bv9sdn0e9dz2vdpzwx" timestamp="0">28</key></foreign-keys><ref-type

name="Report">27</ref-type><contributors><authors><author>RTI
International,</author></authors><tertiary-authors><author>National Toxicology
Program</author></tertiary-authors></contributors><titles><title>Diethyl phthalate: Reproduction and
fertility assessment in CD-1 mice when administered in the
feed</title></titles><dates><year>1984</year></dates><pub-location>Research Triangle Park,
NC</pub-location><publisher><style face="normal" font="default" size="10">RTI
International</style></publisher><label>1313352</label><urls><related-
urls><url>https://heronet.epa.gov/heronet/index.cfm/reference/download/reference_id/1313352C3 -
1097,2305</url></related-urls></urls><language>English</language></record></Cite></EndNote>],
and in one *low confidence* study that evaluated effects in F1 female rats that had been exposed to a low
dose of DEP (0.173 mg/kg-day) since birth [ADDIN EN.CITE
<EndNote><Cite><Author>Manservisi</Author><Year>2015</Year><RecNum>48</RecNum><DisplayTe
xt>(Manservisi et al. 2015)</DisplayText><record><rec-number>48</rec-number><foreign-keys><key
app="EN" db-id="rpv9f5v5cfrzxgesv5bv9sdn0e9dz2vdpzwx" timestamp="0">48</key></foreign-
keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Manservisi,
F.</author><author>Gopalakrishnan, K.</author><author>Tibaldi, E.</author><author>Hysi,
A.</author><author>Iezzi, M.</author><author>Lambertini, L.</author><author>Teitelbaum,
S.</author><author>Chen, J.</author><author>Belpoggi,
F.</author></authors></contributors><titles><title>Effect of maternal exposure to endocrine disrupting
chemicals on reproduction and mammary gland development in female Sprague-Dawley
rats</title><secondary-title>Reproductive Toxicology</secondary-title><alt-title>Reprod Toxicol</alt-
title><short-title>Reproductive Toxicology</short-title></titles><pages>110-
119</pages><volume>54</volume><dates><year>2015</year></dates><isbn>ISSN 0890-
6238
EISSN 1873-1708</isbn><accession-num>25554385</accession-
num><label>3230541</label><urls><related-
urls><url>https://heronet.epa.gov/heronet/index.cfm/reference/download/reference_id/3230541C3 -
1097,2245</url></related-urls></urls><custom1>false</custom1><electronic-resource-
num>10.1016/j.reprotox.2014.12.013</electronic-resource-
num><language>English</language></record></Cite></EndNote>]. In the two-generation study in rats,
[HYPERLINK \l "_ENREF_10" \o "Fujii, 2005 #22"] observed that F1 parental females had a slight but
statistically significant decrease in gestation length after exposure to 1375 mg/kg-day DEP. This effect
was not observed in F0 parental females, and there were no effects on copulation, fertility, or litter size
in either generation. In the continuous breeding study in mice [ADDIN EN.CITE
<EndNote><Cite><Author>RTI
International</Author><Year>1984</Year><RecNum>28</RecNum><DisplayText>(RTI International
1984)</DisplayText><record><rec-number>28</rec-number><foreign-keys><key app="EN" db-
id="rpv9f5v5cfrzxgesv5bv9sdn0e9dz2vdpzwx" timestamp="0">28</key></foreign-keys><ref-type
name="Report">27</ref-type><contributors><authors><author>RTI
International,</author></authors><tertiary-authors><author>National Toxicology
Program</author></tertiary-authors></contributors><titles><title>Diethyl phthalate: Reproduction and
fertility assessment in CD-1 mice when administered in the
feed</title></titles><dates><year>1984</year></dates><pub-location>Research Triangle Park,
NC</pub-location><publisher><style face="normal" font="default" size="10">RTI
International</style></publisher><label>1313352</label><urls><related-

urls><url>https://heronet.epa.gov/heronet/index.cfm/reference/download/reference_id/1313352C3 - 1097,2305</url></related-urls></urls><language>English</language></record></Cite></EndNote>], litter size was statistically significantly reduced by 14% in F1 parental females following exposure to 3640 mg/kg-day. There were no effects on litter size or number of litters per mating pair for F0 parental females, and no effect on fertility (number fertile per number cohabited) in either F0 or F1 animals. [HYPERLINK \l "_ENREF_29" \o "Manservisi, 2015 #48"] found that pregnancy rate was not affected, and that litter size was statistically significantly increased in DEP-treated females compared to controls, although the finding for litter size is considered *low confidence* due to the concerns described in Section 3.2. Given that some effects on litter size and gestation length in F1 parental females were observed in two *high confidence* studies, the evidence for effects on pregnancy outcomes was considered *slight*.

Maternal body weight parameters were assessed in the two-generation study in rats by [HYPERLINK \l "_ENREF_10" \o "Fujii, 2005 #22"] and in several studies that exposed animals during gestation only. In rats exposed to DEP from GD 6-15, [HYPERLINK \l "_ENREF_34" \o "NTP, 1988 #29"] reported a decreasing trend in maternal body weight gain after correcting for gravid uterine weight, indicating that the effect was maternal rather than fetal. In contrast, the multigenerational exposure study by [HYPERLINK \l "_ENREF_10" \o "Fujii, 2005 #22"] reported a statistically significant increase in F0 maternal body weight gain in several dose groups, but no effects on maternal weight gain in the F1 generation. No effects on maternal weight gain were observed in the remaining studies in mice [ADDIN EN.CITE <EndNote><Cite><Author>Hardin</Author><Year>1987</Year><RecNum>3</RecNum><DisplayText>(Hardin et al. 1987)</DisplayText><record><rec-number>3</rec-number><foreign-keys><key app="EN" db-id="rpv9f5v5cfrzxgesv5bv9sdn0e9dz2vdpzwx" timestamp="0">3</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Hardin, B. D.</author><author>Schuler, R. L.</author><author>Burg, , J. R.</author><author>Booth, G. M.</author><author>Hazelden, K. P.</author><author>Mackenzie, K. M.</author><author>Piccirillo, V. J.</author><author>Smith, K. N.</author></authors></contributors><titles><title>Evaluation of 60 chemicals in a preliminary developmental toxicity test</title><secondary-title>Teratogenesis, Carcinogenesis, and Mutagenesis</secondary-title><alt-title>Teratog Carcinog Mutagen</alt-title><short-title>Teratogenesis, Carcinogenesis, and Mutagenesis</short-title></titles><pages>29-48</pages><volume>7</volume><number>1</number><dates><year>1987</year></dates><isbn>ISSN 0270-3211
EISSN 1520-6866</isbn><accession-num>2884741</accession-num><label>62212</label><urls><related-urls><url>https://heronet.epa.gov/heronet/index.cfm/reference/download/reference_id/62212C3 - 367,1097,1666,1668,1803,1826,1905,1906,1913,1926,2116,2146,2148,2188,2195,2205,2206,2227,2262,2265,2269,2272,2273,2294,2305,2320,2485,2664,2699</url></related-urls></urls><custom1>true</custom1><electronic-resource-num>10.1002/tcm.1770070106</electronic-resource-num><language>English</language></record></Cite></EndNote>], rats [ADDIN EN.CITE ADDIN EN.CITE.DATA], or rabbits [ADDIN EN.CITE <EndNote><Cite><Author>Procter & Gamble</Author><Year>1994</Year><RecNum>32</RecNum><DisplayText>(Procter & Gamble 1994)</DisplayText><record><rec-number>32</rec-number><foreign-keys><key app="EN" db-id="rpv9f5v5cfrzxgesv5bv9sdn0e9dz2vdpzwx" timestamp="0">32</key></foreign-keys><ref-type name="Report">27</ref-type><contributors><authors><author>Procter & Gamble,</author></authors></contributors><titles><title>Teratogenicity study of E-2426.01 (diethyl

phthalate) by dermal application to rabbits with cover letter dated 05/02/94</title></titles><dates><year>1994</year></dates><pub-location>Washington, DC</pub-location><publisher>U.S. Environmental Protection Agency</publisher><isbn>8EHQ-86940000362</isbn><label>1315778</label><urls><related-urls><url>https://heronet.epa.gov/heronet/index.cfm/reference/download/reference_id/1315778C3 - 1097,2305</url></related-urls></urls><language>English</language><modified-date>Proctor & Gamble Company</modified-date></record></Cite></EndNote>], although the latter study should be interpreted with additional caution, since maternal body weight during pregnancy can be highly variable in rabbits [ADDIN EN.CITE <EndNote><Cite><Author>US EPA</Author><Year>1991</Year><RecNum>65</RecNum><DisplayText>(US EPA 1991)</DisplayText><record><rec-number>65</rec-number><foreign-keys><key app="EN" db-id="rpv9f5v5cfrzxgesv5bv9sdn0e9dz2vdpzwx" timestamp="0">65</key></foreign-keys><ref-type name="Government Document">46</ref-type><contributors><authors><author>US EPA,</author></authors><secondary-authors><author>Risk Assessment Forum</author></secondary-authors></contributors><titles><title>Guidelines for developmental toxicity risk assessment.</title></titles><dates><year>1991</year></dates><pub-location>Washington DC</pub-location><publisher>U.S. Environmental Protecion Agency</publisher><isbn>EPA/600/FR-91/001</isbn><urls></urls></record></Cite></EndNote>]. Taken together, DEP may affect maternal weight gain at high doses in the rat, but effects were inconsistent, possibly due to interspecies sensitivity differences or differences in experimental design. Therefore, the evidence for effects on maternal body weight gain are considered *indeterminate*.

Multiple studies evaluated organ weights in females that had been exposed to DEP during development or as adults. No DEP-related effects were reported for gravid uterine weights in adult pregnant rats or rabbits [ADDIN EN.CITE <EndNote><Cite><Author>NTP</Author><Year>1988</Year><RecNum>29</RecNum><DisplayText>(NTP 1988; Procter & Gamble 1994)</DisplayText><record><rec-number>29</rec-number><foreign-keys><key app="EN" db-id="rpv9f5v5cfrzxgesv5bv9sdn0e9dz2vdpzwx" timestamp="0">29</key></foreign-keys><ref-type name="Report">27</ref-type><contributors><authors><author>NTP,</author></authors></contributors><titles><title>Developmental toxicity evaluation of diethyl phthalate (CAS No. 84-66-2) administered to CD rats on gestational days 6 through 15</title></titles><dates><year>1988</year></dates><pub-location>Research Triangle Park, NC
</pub-location><isbn>NTP-88-336; RTI-207</isbn><label>1313353</label><urls><related-urls><url>https://heronet.epa.gov/heronet/index.cfm/reference/download/reference_id/1313353C3 - 1097,2305</url></related-urls></urls><language>English</language><modified-date>National Toxicology Program</modified-date></record></Cite><Cite><Author>Procter & Gamble</Author><Year>1994</Year><RecNum>32</RecNum><record><rec-number>32</rec-number><foreign-keys><key app="EN" db-id="rpv9f5v5cfrzxgesv5bv9sdn0e9dz2vdpzwx" timestamp="0">32</key></foreign-keys><ref-type name="Report">27</ref-type><contributors><authors><author>Procter & Gamble,</author></authors></contributors><titles><title>Teratogenicity study of E-2426.01 (diethyl phthalate) by dermal application to rabbits with cover letter dated 05/02/94</title></titles><dates><year>1994</year></dates><pub-location>Washington, DC</pub-location><publisher>U.S. Environmental Protection Agency</publisher><isbn>8EHQ-

86940000362</isbn><label>1315778</label><urls><related-urls><url>https://heronet.epa.gov/heronet/index.cfm/reference/download/reference_id/1315778C3 -1097,2305</url></related-urls></urls><language>English</language><modified-date>Proctor & Gamble Company</modified-date></record></Cite></EndNote>] or absolute or relative uterine or ovarian weights in adult rats [ADDIN EN.CITE ADDIN EN.CITE.DATA], F0 or F1 adult rats [ADDIN EN.CITE <EndNote><Cite><Author>Fujii</Author><Year>2005</Year><RecNum>22</RecNum><DisplayText>(Fujii et al. 2005)</DisplayText><record><rec-number>22</rec-number><foreign-keys><key app="EN" db-id="rpv9f5v5cfrzxgesv5bv9sdn0e9dz2vdpzwx" timestamp="0">22</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Fujii, S.</author><author>Yabe, K.</author><author>Furukawa, M.</author><author>Hirata, M.</author><author>Kiguchi, M.</author><author>Ikka, T.</author></authors></contributors><titles><title>A two-generation reproductive toxicity study of diethyl phthalate (DEP) in rats</title><secondary-title>Journal of Toxicological Sciences</secondary-title><alt-title>J Toxicol Sci</alt-title><short-title>Journal of Toxicological Sciences</short-title></titles><pages>S97-116</pages><volume>30</volume><number>Special Issue P</number><dates><year>2005</year></dates><isbn>ISSN 0388-1350
EISSN 1880-3989</isbn><accession-num>16641546</accession-num><label>1298274</label><urls><related-urls><url>https://heronet.epa.gov/heronet/index.cfm/reference/download/reference_id/1298274C3 -1097,2187,2212,2245,2294,2305,2344</url></related-urls></urls><custom1>true</custom1><electronic-resource-num>10.2131/jts.30.S97</electronic-resource-num><language>English</language></record></Cite></EndNote>], or F1 adult mice [ADDIN EN.CITE <EndNote><Cite><Author>RTI International</Author><Year>1984</Year><RecNum>28</RecNum><DisplayText>(RTI International 1984)</DisplayText><record><rec-number>28</rec-number><foreign-keys><key app="EN" db-id="rpv9f5v5cfrzxgesv5bv9sdn0e9dz2vdpzwx" timestamp="0">28</key></foreign-keys><ref-type name="Report">27</ref-type><contributors><authors><author>RTI International,</author></authors><tertiary-authors><author>National Toxicology Program</author></tertiary-authors></contributors><titles><title>Diethyl phthalate: Reproduction and fertility assessment in CD-1 mice when administered in the feed</title></titles><dates><year>1984</year></dates><pub-location>Research Triangle Park, NC</pub-location><publisher><style face="normal" font="default" size="10">RTI International</style></publisher><label>1313352</label><urls><related-urls><url>https://heronet.epa.gov/heronet/index.cfm/reference/download/reference_id/1313352C3 -1097,2305</url></related-urls></urls><language>English</language></record></Cite></EndNote>]. In contrast, absolute uterine weights were statistically significantly reduced in F1 and F2 weanling females after gestational exposure to 1297 mg/kg-day and 1375 mg/kg-day, respectively, although this effect appears to be transient, since effects on uterine weight were not reported in adult F1 females at necropsy [ADDIN EN.CITE <EndNote><Cite><Author>Fujii</Author><Year>2005</Year><RecNum>22</RecNum><DisplayText>(Fujii et al. 2005)</DisplayText><record><rec-number>22</rec-number><foreign-keys><key app="EN" db-id="rpv9f5v5cfrzxgesv5bv9sdn0e9dz2vdpzwx" timestamp="0">22</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Fujii, S.</author><author>Yabe, K.</author><author>Furukawa, M.</author><author>Hirata, M.</author><author>Kiguchi,

M.</author><author>Ikka, T.</author></authors></contributors><titles><title>A two-generation reproductive toxicity study of diethyl phthalate (DEP) in rats</title><secondary-title>Journal of Toxicological Sciences</secondary-title><alt-title>J Toxicol Sci</alt-title><short-title>Journal of Toxicological Sciences</short-title></titles><pages>S97-116</pages><volume>30</volume><number>Special Issue P</number><dates><year>2005</year></dates><isbn>ISSN 0388-1350
EISSN 1880-3989</isbn><accession-num>16641546</accession-num><label>1298274</label><urls><related-urls><url>https://heronet.epa.gov/heronet/index.cfm/reference/download/reference_id/1298274C3 -1097,2187,2212,2245,2294,2305,2344</url></related-urls></urls><custom1>true</custom1><electronic-resource-num>10.2131/jts.30.S97</electronic-resource-num><language>English</language></record></Cite></EndNote>]. Statistically significant increases in relative ovary weights in F0 and F1 female rats following exposure to 2.85 mg/kg-day and 1.425 mg/kg-day, respectively, were reported in the multigenerational study by [HYPERLINK \l "_ENREF_41" \o "Pereira, 2007 #38"], although this study is considered *low confidence* due to the concerns discussed in Section 3.2. Taken together, while there is some evidence for effects on various organ weights from both *high* and *low* confidence studies, most studies do not support that DEP induces permanent, adverse effects. The evidence for effects on female reproductive organ weight is considered *slight*.

In a histopathological evaluation, the *low confidence* study by [HYPERLINK \l "_ENREF_29" \o "Manservisi, 2015 #48"] reported mammary gland effects (decreases in the size of lobular structures and a darker and denser appearance of these structures due to the lower dilation of the secretory alveoli) in parous F1 females that had been exposed to 0.173 mg/kg-day DEP since birth, although the sample size was small (n=3/group) and only semi-quantitative results were presented; corresponding effects in nulliparous females after lactational and direct DEP exposure were not observed. Other studies that evaluated gross and histopathological alterations in ovaries, uteri, vaginas, or mammary glands did not observe DEP-induced effects [ADDIN EN.CITE   ADDIN EN.CITE.DATA]. Since effects were only observed in one *low confidence* study, the evidence for histopathological effects on female reproductive organs is considered *indeterminate*.

In adolescent F1 female rats exposed to DEP at 1375 mg/kg-day, the onset of puberty as measured by the age at vaginal opening was delayed by 6% compared to control [ADDIN EN.CITE <EndNote><Cite><Author>Fujii</Author><Year>2005</Year><RecNum>22</RecNum><DisplayText>(Fujii et al. 2005)</DisplayText><record><rec-number>22</rec-number><foreign-keys><key app="EN" db-id="rpv9f5v5cfrzxgesv5bv9sdn0e9dz2vdpzwx" timestamp="0">22</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Fujii, S.</author><author>Yabe, K.</author><author>Furukawa, M.</author><author>Hirata, M.</author><author>Kiguchi, M.</author><author>Ikka, T.</author></authors></contributors><titles><title>A two-generation reproductive toxicity study of diethyl phthalate (DEP) in rats</title><secondary-title>Journal of Toxicological Sciences</secondary-title><alt-title>J Toxicol Sci</alt-title><short-title>Journal of Toxicological Sciences</short-title></titles><pages>S97-116</pages><volume>30</volume><number>Special Issue P</number><dates><year>2005</year></dates><isbn>ISSN 0388-1350
EISSN 1880-3989</isbn><accession-num>16641546</accession-num><label>1298274</label><urls><related-urls><url>https://heronet.epa.gov/heronet/index.cfm/reference/download/reference_id/1298274C3 -

1097,2187,2212,2245,2294,2305,2344</url></related-urls></urls><custom1>true</custom1><electronic-resource-num>10.2131/jts.30.S97</electronic-resource-num><language>English</language></record></Cite></EndNote>]. Animals in this dose group had decreased growth in early postnatal life (discussed in Section 3.5.1) but had reached similar body weights compared to controls at the time puberty was attained, which suggests that the delay in puberty was related to delayed growth. AGD in F1 or F2 female pups was not affected in this study [ADDIN EN.CITE <EndNote><Cite><Author>Fujii</Author><Year>2005</Year><RecNum>22</RecNum><DisplayText>(Fujii et al. 2005)</DisplayText><record><rec-number>22</rec-number><foreign-keys><key app="EN" db-id="rpv9f5v5cfrzxgesv5bv9sdn0e9dz2vdpzwx" timestamp="0">22</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Fujii, S.</author><author>Yabe, K.</author><author>Furukawa, M.</author><author>Hirata, M.</author><author>Kiguchi, M.</author><author>Ikka, T.</author></authors></contributors><titles><title>A two-generation reproductive toxicity study of diethyl phthalate (DEP) in rats</title><secondary-title>Journal of Toxicological Sciences</secondary-title><alt-title>J Toxicol Sci</alt-title><short-title>Journal of Toxicological Sciences</short-title></titles><pages>S97-116</pages><volume>30</volume><number>Special Issue P</number><dates><year>2005</year></dates><isbn>ISSN 0388-1350
EISSN 1880-3989</isbn><accession-num>16641546</accession-num><label>1298274</label><urls><related-urls><url>https://heronet.epa.gov/heronet/index.cfm/reference/download/reference_id/1298274C3 -1097,2187,2212,2245,2294,2305,2344</url></related-urls></urls><custom1>true</custom1><electronic-resource-num>10.2131/jts.30.S97</electronic-resource-num><language>English</language></record></Cite></EndNote>] or in F1 females in the study by [HYPERLINK \l "_ENREF_12" \o "Gray, 2000 #10"]. There were no effects on estrous cyclicity in F0 or F1 females [ADDIN EN.CITE <EndNote><Cite><Author>Fujii</Author><Year>2005</Year><RecNum>22</RecNum><DisplayText>(Fujii et al. 2005)</DisplayText><record><rec-number>22</rec-number><foreign-keys><key app="EN" db-id="rpv9f5v5cfrzxgesv5bv9sdn0e9dz2vdpzwx" timestamp="0">22</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Fujii, S.</author><author>Yabe, K.</author><author>Furukawa, M.</author><author>Hirata, M.</author><author>Kiguchi, M.</author><author>Ikka, T.</author></authors></contributors><titles><title>A two-generation reproductive toxicity study of diethyl phthalate (DEP) in rats</title><secondary-title>Journal of Toxicological Sciences</secondary-title><alt-title>J Toxicol Sci</alt-title><short-title>Journal of Toxicological Sciences</short-title></titles><pages>S97-116</pages><volume>30</volume><number>Special Issue P</number><dates><year>2005</year></dates><isbn>ISSN 0388-1350
EISSN 1880-3989</isbn><accession-num>16641546</accession-num><label>1298274</label><urls><related-urls><url>https://heronet.epa.gov/heronet/index.cfm/reference/download/reference_id/1298274C3 -1097,2187,2212,2245,2294,2305,2344</url></related-urls></urls><custom1>true</custom1><electronic-resource-num>10.2131/jts.30.S97</electronic-resource-num><language>English</language></record></Cite></EndNote>]. Likewise, [HYPERLINK \l "_ENREF_57" \o "Shiraishi, 2006 #30"] evaluated serum hormone levels in adult females exposed to DEP for 28 days and found no effects on estradiol, testosterone, or gonadotropins. Taken together, evidence for effects on female morphological development, estrous cyclicity, and hormones were all considered to be *indeterminate*.

3.4.2. *Synthesis of results for female reproductive effects*

The available studies suggest there is *slight* evidence that DEP is a female reproductive toxicant (Table 3). Some statistically significant effects were reported for decreased gestation length, decreased litter size, maternal body weight gain, organ weight changes, and age at puberty from two *high confidence* multi-generational studies in rats and mice. Effects on gestation length [ADDIN EN.CITE <EndNote><Cite><Author>Fujii</Author><Year>2005</Year><RecNum>22</RecNum><DisplayText>(Fujii et al. 2005)</DisplayText><record><rec-number>22</rec-number><foreign-keys><key app="EN" db-id="rpv9f5v5cfrzxgesv5bv9sdn0e9dz2vdpzwx" timestamp="0">22</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Fujii, S.</author><author>Yabe, K.</author><author>Furukawa, M.</author><author>Hirata, M.</author><author>Kiguchi, M.</author><author>Ikka, T.</author></authors></contributors><titles><title>A two-generation reproductive toxicity study of diethyl phthalate (DEP) in rats</title><secondary-title>Journal of Toxicological Sciences</secondary-title><alt-title>J Toxicol Sci</alt-title><short-title>Journal of Toxicological Sciences</short-title></titles><pages>S97-116</pages><volume>30</volume><number>Special Issue P</number><dates><year>2005</year></dates><isbn>ISSN 0388-1350
EISSN 1880-3989</isbn><accession-num>16641546</accession-num><label>1298274</label><urls><related-urls><url>https://heronet.epa.gov/heronet/index.cfm/reference/download/reference_id/1298274C3 - 1097,2187,2212,2245,2294,2305,2344</url></related-urls></urls><custom1>true</custom1><electronic-resource-num>10.2131/jts.30.S97</electronic-resource-num><language>English</language></record></Cite></EndNote>] and litter size [ADDIN EN.CITE <EndNote><Cite><Author>RTI International</Author><Year>1984</Year><RecNum>28</RecNum><DisplayText>(RTI International 1984)</DisplayText><record><rec-number>28</rec-number><foreign-keys><key app="EN" db-id="rpv9f5v5cfrzxgesv5bv9sdn0e9dz2vdpzwx" timestamp="0">28</key></foreign-keys><ref-type name="Report">27</ref-type><contributors><authors><author>RTI International,</author></authors><tertiary-authors><author>National Toxicology Program</author></tertiary-authors></contributors><titles><title>Diethyl phthalate: Reproduction and fertility assessment in CD-1 mice when administered in the feed</title></titles><dates><year>1984</year></dates><pub-location>Research Triangle Park, NC</pub-location><publisher><style face="normal" font="default" size="10">RTI International</style></publisher><label>1313352</label><urls><related-urls><url>https://heronet.epa.gov/heronet/index.cfm/reference/download/reference_id/1313352C3 - 1097,2305</url></related-urls></urls><language>English</language></record></Cite></EndNote>] in these studies were observed in F1 parental animals but not in F0, possibly suggesting that the F1 animals may have increased sensitivity due to their developmental exposure to DEP; however, in all cases, the magnitude of effect was small and was observed only at high dose levels. Otherwise, with the exception of some organ weight and histopathological changes in *low* confidence studies, results across studies were largely negative. It is possible that differences in test animal species/strains and experimental designs may contribute to some of these inconsistencies across studies.

3.5. Developmental effects

Figures indicating the doses at which statistically significant developmental effects occurred are provided in the Supplementary Materials (Figures S8 – S12 and S18).

3.5.1. Summary of available studies

Four studies evaluated fetal survival following gestational exposure to DEP [ADDIN EN.CITE ADDIN EN.CITE.DATA], and four studies evaluated the number of live pups at birth following gestational [ADDIN EN.CITE ADDIN EN.CITE.DATA] or multigenerational [ADDIN EN.CITE ADDIN EN.CITE.DATA] exposure to DEP. Of these, only the continuous breeding study in CD-1 mice [ADDIN EN.CITE <EndNote><Cite><Author>RTI International</Author><Year>1984</Year><RecNum>28</RecNum><DisplayText>(RTI International 1984)</DisplayText><record><rec-number>28</rec-number><foreign-keys><key app="EN" db-id="rpv9f5v5cfrzxgesv5bv9sdn0e9dz2vdpzwx" timestamp="0">28</key></foreign-keys><ref-type name="Report">27</ref-type><contributors><authors><author>RTI International,</author></authors><tertiary-authors><author>National Toxicology Program</author></tertiary-authors></contributors><titles><title>Diethyl phthalate: Reproduction and fertility assessment in CD-1 mice when administered in the feed</title></titles><dates><year>1984</year></dates><pub-location>Research Triangle Park, NC</pub-location><publisher><style face="normal" font="default" size="10">RTI International</style></publisher><label>1313352</label><urls><related-urls><url>https://heronet.epa.gov/heronet/index.cfm/reference/download/reference_id/1313352C3 - 1097,2305</url></related-urls></urls><language>English</language></record></Cite></EndNote>] observed a dose-related effect. The number of live F2 offspring at birth (males and females combined) was statistically significantly decreased by 14% in the 3640 mg/kg-day DEP exposure group relative to controls, whereas no effects on survival were observed in the F1 offspring. In a gestational exposure study in Sprague-Dawley rats, [HYPERLINK \l "_ENREF_20" \o "Howdeshell, 2008 #8"] reported a statistically significant increase in resorptions and fetal mortality at 600 mg/kg-day DEP, but this effect was not observed in any other higher or lower DEP dose groups and thus did not appear to be treatment-related. Otherwise, in the rat two-generation study and in the remaining four gestational exposure studies in rats, mice, and rabbits, there were no effects on the number of implantations or resorptions [ADDIN EN.CITE ADDIN EN.CITE.DATA], viability of fetuses [ADDIN EN.CITE ADDIN EN.CITE.DATA], or viability of pups at birth [ADDIN EN.CITE ADDIN EN.CITE.DATA]. There was no effect on offspring sex ratio in any of the studies that assessed this endpoint, which included studies in rats, mice, and rabbits [ADDIN EN.CITE ADDIN EN.CITE.DATA]. Given the evidence of decreased fetal survival in the *high confidence* continuous breeding study in mice but lack of effect in all other studies, the evidence for DEP effects on fetal survival following gestational exposure is considered *indeterminate*.

The three studies that reported postnatal survival found differing results. In F1 female rats exposed to a low dose of DEP (0.173 mg/kg-day) from birth (PND 1) through sacrifice, there was a statistically significant increase in the postnatal mortality of F2 pups through PND 14 [ADDIN EN.CITE <EndNote><Cite><Author>Manservisi</Author><Year>2015</Year><RecNum>48</RecNum><DisplayText>(Manservisi et al. 2015)</DisplayText><record><rec-number>48</rec-number><foreign-keys><key app="EN" db-id="rpv9f5v5cfrzxgesv5bv9sdn0e9dz2vdpzwx" timestamp="0">48</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Manservisi, F.</author><author>Gopalakrishnan, K.</author><author>Tibaldi, E.</author><author>Hysi, A.</author><author>Iezzi, M.</author><author>Lambertini, L.</author><author>Teitelbaum, S.</author><author>Chen, J.</author><author>Belpoggi, F.</author></authors></contributors><titles><title>Effect of maternal exposure to endocrine disrupting chemicals on reproduction and mammary gland development in female Sprague-Dawley rats</title><secondary-title>Reproductive Toxicology</secondary-title><alt-title>Reprod Toxicol</alt-title><short-title>Reproductive Toxicology</short-title></titles><pages>110-119</pages><volume>54</volume><dates><year>2015</year></dates><isbn>ISSN 0890-6238
EISSN 1873-1708</isbn><accession-num>25554385</accession-num><label>3230541</label><urls><related-urls><url>https://heronet.epa.gov/heronet/index.cfm/reference/download/reference_id/3230541C3 - 1097,2245</url></related-urls></urls><custom1>false</custom1><electronic-resource-num>10.1016/j.reprotox.2014.12.013</electronic-resource-num><language>English</language></record></Cite></EndNote>], although this result is considered *low confidence* due to the concerns discussed in Section 3.2. In contrast, no significant effects were observed in *high confidence* studies. The two-generation study in rats from [HYPERLINK \l "_ENREF_10" \o "Fujii, 2005 #22"] indicated a non-significant trend towards lower survival of F1 offspring at PND 21 in the DEP treatment groups, but this trend was not observed in F2 offspring and it is not clear that it is treatment-related. There was no effect on F1 offspring viability through PND 3 in mice exposed to 4,500 mg/kg-day DEP from GD 6-13 [ADDIN EN.CITE <EndNote><Cite><Author>Hardin</Author><Year>1987</Year><RecNum>3</RecNum><DisplayText>(Hardin et al. 1987)</DisplayText><record><rec-number>3</rec-number><foreign-keys><key app="EN" db-id="rpv9f5v5cfrzxgesv5bv9sdn0e9dz2vdpzwx" timestamp="0">3</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Hardin, B. D.</author><author>Schuler, R. L.</author><author>Burg, , J. R.</author><author>Booth, G. M.</author><author>Hazelden, K. P.</author><author>Mackenzie, K. M.</author><author>Piccirillo, V. J.</author><author>Smith, K. N.</author></authors></contributors><titles><title>Evaluation of 60 chemicals in a preliminary developmental toxicity test</title><secondary-title>Teratogenesis, Carcinogenesis, and Mutagenesis</secondary-title><alt-title>Teratog Carcinog Mutagen</alt-title><short-title>Teratogenesis, Carcinogenesis, and Mutagenesis</short-title></titles><pages>29-48</pages><volume>7</volume><number>1</number><dates><year>1987</year></dates><isbn>ISSN 0270-3211
EISSN 1520-6866</isbn><accession-num>2884741</accession-num><label>62212</label><urls><related-urls><url>https://heronet.epa.gov/heronet/index.cfm/reference/download/reference_id/62212C3 - 367,1097,1666,1668,1803,1826,1905,1906,1913,1926,2116,2146,2148,2188,2195,2205,2206,2227,2262,2265,2269,2272,2273,2294,2305,2320,2485,2664,2699</url></related-urls></urls><custom1>true</custom1><electronic-resource-num>10.1002/tcm.1770070106</electronic-resource-num><language>English</language></record></Cite></EndNote>]. Taken together, the evidence for the effects of DEP on postnatal survival is considered *indeterminate*.

No effects on fetal growth were observed in six studies conducted in rats, mice, and rabbits, all of which were considered *high confidence* for this outcome. The two-generation reproductive study in rats [ADDIN EN.CITE <EndNote><Cite><Author>Fujii</Author><Year>2005</Year><RecNum>22</RecNum><DisplayText>(Fujii et al. 2005)</DisplayText><record><rec-number>22</rec-number><foreign-keys><key app="EN" db-

id="rpv9f5v5cfrzxgesv5bv9sdn0e9dz2vdpzwx" timestamp="0">22</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Fujii, S.</author><author>Yabe, K.</author><author>Furukawa, M.</author><author>Hirata, M.</author><author>Kiguchi, M.</author><author>Ikka, T.</author></authors></contributors><titles><title>A two-generation reproductive toxicity study of diethyl phthalate (DEP) in rats</title><secondary-title>Journal of Toxicological Sciences</secondary-title><alt-title>J Toxicol Sci</alt-title><short-title>Journal of Toxicological Sciences</short-title></titles><pages>S97-116</pages><volume>30</volume><number>Special Issue P</number><dates><year>2005</year></dates><isbn>ISSN 0388-1350
EISSN 1880-3989</isbn><accession-num>16641546</accession-num><label>1298274</label><urls><related-urls><url>https://heronet.epa.gov/heronet/index.cfm/reference/download/reference_id/1298274C3 - 1097,2187,2212,2245,2294,2305,2344</url></related-urls></urls><custom1>true</custom1><electronic-resource-num>10.2131/jts.30.S97</electronic-resource-num><language>English</language></record></Cite></EndNote>] and the continuous breeding study in mice [ADDIN EN.CITE <EndNote><Cite><Author>RTI International</Author><Year>1984</Year><RecNum>28</RecNum><DisplayText>(RTI International 1984)</DisplayText><record><rec-number>28</rec-number><foreign-keys><key app="EN" db-id="rpv9f5v5cfrzxgesv5bv9sdn0e9dz2vdpzwx" timestamp="0">28</key></foreign-keys><ref-type name="Report">27</ref-type><contributors><authors><author>RTI International,</author></authors><tertiary-authors><author>National Toxicology Program</author></tertiary-authors></contributors><titles><title>Diethyl phthalate: Reproduction and fertility assessment in CD-1 mice when administered in the feed</title></titles><dates><year>1984</year></dates><pub-location>Research Triangle Park, NC</pub-location><publisher><style face="normal" font="default" size="10">RTI International</style></publisher><label>1313352</label><urls><related-urls><url>https://heronet.epa.gov/heronet/index.cfm/reference/download/reference_id/1313352C3 - 1097,2305</url></related-urls></urls><language>English</language></record></Cite></EndNote>] both reported that F1 and F2 offspring body weights at birth were similar between DEP-treated animals and controls. Likewise, three gestational exposure studies indicated that fetal body weights of rats and rabbits [ADDIN EN.CITE <EndNote><Cite><Author>NTP</Author><Year>1988</Year><RecNum>29</RecNum><DisplayText>(NTP 1988; Procter & Gamble 1994)</DisplayText><record><rec-number>29</rec-number><foreign-keys><key app="EN" db-id="rpv9f5v5cfrzxgesv5bv9sdn0e9dz2vdpzwx" timestamp="0">29</key></foreign-keys><ref-type name="Report">27</ref-type><contributors><authors><author>NTP,</author></authors></contributors><titles><title>Developmental toxicity evaluation of diethyl phthalate (CAS No. 84-66-2) administered to CD rats on gestational days 6 through 15</title></titles><dates><year>1988</year></dates><pub-location>Research Triangle Park, NC
</pub-location><isbn>NTP-88-336; RTI-207</isbn><label>1313353</label><urls><related-urls><url>https://heronet.epa.gov/heronet/index.cfm/reference/download/reference_id/1313353C3 - 1097,2305</url></related-urls></urls><language>English</language><modified-date>National Toxicology Program</modified-date></record></Cite><Cite><Author>Procter & Gamble</Author><Year>1994</Year><RecNum>32</RecNum><record><rec-number>32</rec-number><foreign-keys><key app="EN" db-id="rpv9f5v5cfrzxgesv5bv9sdn0e9dz2vdpzwx"

timestamp="0">32</key></foreign-keys><ref-type name="Report">27</ref-type><contributors><authors><author>Procter & Gamble,</author></authors></contributors><titles><title>Teratogenicity study of E-2426.01 (diethyl phthalate) by dermal application to rabbits with cover letter dated 05/02/94</title></titles><dates><year>1994</year></dates><pub-location>Washington, DC</pub-location><publisher>U.S. Environmental Protection Agency</publisher><isbn>8EHQ-86940000362</isbn><label>1315778</label><urls><related-urls><url>https://heronet.epa.gov/heronet/index.cfm/reference/download/reference_id/1315778C3 -1097,2305</url></related-urls></urls><language>English</language><modified-date>Proctor & Gamble Company</modified-date></record></Cite></EndNote>] or body weights of rats or mice at birth [ADDIN EN.CITE ADDIN EN.CITE.DATA] were similar between DEP-treated animals and controls. While this data suggests that DEP does not impact fetal growth across multiple species, it was decided among reviewers that there were not enough studies available to support a judgement of *compelling evidence of no effect*. The evidence for effects of DEP on prenatal growth is therefore considered *indeterminate*.

Conversely, decreased postnatal growth was reported in multiple studies that evaluated this endpoint. In the *high confidence* two-generation reproduction study, [HYPERLINK \l "_ENREF_10" \o "Fujii, 2005 #22"] observed that F1 and F2 Sprague-Dawley rat offspring exposed to 1,297 mg/kg-day and 1,375 mg/kg-day DEP, respectively, had lower body weights relative to controls at PND 4, 7, 14, and 21. The decrease in body weight was significant for F1 female pups at all time points, and for F1 and F2 male pups and F2 female pups at PND 21. These pups also had a delay in pinna detachment (a developmental biomarker) relative to controls, which was statistically significant for F1 males. Similarly, the *high confidence* continuous breeding study in mice by [HYPERLINK \l "_ENREF_54" \o "RTI International, 1984 #28"] found that F1 male and female pup body weights at the time of weaning (PND 21) were lower in DEP-treated groups (3,640 mg/kg-day) compared to controls and remained lower than controls at the time of mating (PND 74 ± 10), although the authors did not perform a statistical analysis. Additionally, the *low confidence* multigenerational studies in rats observed statistically significant reductions in F1 weanling body weights [ADDIN EN.CITE <EndNote><Cite><Author>Pereira</Author><Year>2007</Year><RecNum>18</RecNum><DisplayText>(Pereira and Rao 2007)</DisplayText><record><rec-number>18</rec-number><foreign-keys><key app="EN" db-id="rpv9f5v5cfrzxgesv5bv9sdn0e9dz2vdpzwx" timestamp="0">18</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Pereira, C.</author><author>Rao, C. V.</author></authors></contributors><titles><title>Toxicity study of maternal transfer of polychlorinated biphenyls and diethyl phthalate to 21-day-old male and female weanling pups of Wistar rats</title><secondary-title>Ecotoxicology and Environmental Safety</secondary-title><alt-title>Ecotoxicol Environ Saf</alt-title><short-title>Ecotoxicology and Environmental Safety</short-title></titles><periodical><full-title>Ecotoxicology and Environmental Safety</full-title><abbr-1>Ecotoxicol Environ Saf</abbr-1></periodical><alt-periodical><full-title>Ecotoxicology and Environmental Safety</full-title><abbr-1>Ecotoxicol Environ Saf</abbr-1></alt-periodical><pages>118-125</pages><volume>68</volume><number>1</number><dates><year>2007</year></dates><isbn>ISSN 0147-6513
EISSN 1090-2414</isbn><accession-num>16814384</accession-num><label>789216</label><urls><related-urls><url>https://heronet.epa.gov/heronet/index.cfm/reference/download/reference_id/789216C3 -

384,1097,1826,2212,2245,2294,2305,2370,2669</url></related-urls></urls><custom1>true</custom1><electronic-resource-num>10.1016/j.ecoenv.2006.04.007</electronic-resource-num><language>English</language></record></Cite></EndNote>], F1 adult body weights [ADDIN EN.CITE <EndNote><Cite><Author>Hu</Author><Year>2016</Year><RecNum>47</RecNum><DisplayText>(Hu et al. 2016)</DisplayText><record><rec-number>47</rec-number><foreign-keys><key app="EN" db-id="rpv9f5v5cfrzxgesv5bv9sdn0e9dz2vdpzwx" timestamp="0">47</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Hu, J.</author><author>Raikhel, V.</author><author>Gopalakrishnan, K.</author><author>Fernandez-Hernandez, H.</author><author>Lambertini, L.</author><author>Manservisi, F.</author><author>Falcioni, L.</author><author>Bua, L.</author><author>Belpoggi, F.</author><author>L Teitelbaum, S.</author><author>Chen, J.</author></authors></contributors><titles><title>Effect of postnatal low-dose exposure to environmental chemicals on the gut microbiome in a rodent model</title></titles><pages>26</pages><volume>4</volume><number>1</number><dates><year>2016</year></dates><accession-num>27301250</accession-num><label>3230343</label><urls><related-urls><url>https://heronet.epa.gov/heronet/index.cfm/reference/download/reference_id/3230343C3 - 1097,2206,2245</url></related-urls></urls><custom1>true</custom1><electronic-resource-num>10.1186/s40168-016-0173-2</electronic-resource-num><language>English</language></record></Cite></EndNote>], and F2 body weights at PNDs 7 and 14 [ADDIN EN.CITE <EndNote><Cite><Author>Manservisi</Author><Year>2015</Year><RecNum>48</RecNum><DisplayText>(Manservisi et al. 2015)</DisplayText><record><rec-number>48</rec-number><foreign-keys><key app="EN" db-id="rpv9f5v5cfrzxgesv5bv9sdn0e9dz2vdpzwx" timestamp="0">48</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Manservisi, F.</author><author>Gopalakrishnan, K.</author><author>Tibaldi, E.</author><author>Hysi, A.</author><author>Iezzi, M.</author><author>Lambertini, L.</author><author>Teitelbaum, S.</author><author>Chen, J.</author><author>Belpoggi, F.</author></authors></contributors><titles><title>Effect of maternal exposure to endocrine disrupting chemicals on reproduction and mammary gland development in female Sprague-Dawley rats</title><secondary-title>Reproductive Toxicology</secondary-title><alt-title>Reprod Toxicol</alt-title><short-title>Reproductive Toxicology</short-title></titles><pages>110-119</pages><volume>54</volume><dates><year>2015</year></dates><isbn>ISSN 0890-6238
EISSN 1873-1708</isbn><accession-num>25554385</accession-num><label>3230541</label><urls><related-urls><url>https://heronet.epa.gov/heronet/index.cfm/reference/download/reference_id/3230541C3 - 1097,2245</url></related-urls></urls><custom1>false</custom1><electronic-resource-num>10.1016/j.reprotox.2014.12.013</electronic-resource-num><language>English</language></record></Cite></EndNote>]. There was no effect on F1 offspring growth in the gestational exposure studies in mice by [HYPERLINK \l "_ENREF_15" \o "Hardin, 1987 #3"] (evaluated through PND 3) and in rats by [HYPERLINK \l "_ENREF_12" \o "Gray, 2000 #10"] (evaluated as adults), although these studies used shorter exposure duration (GD 6-13 and GD 14-PND 3, respectively). In peripubertal males exposed to DEP for 7 days, no effects on body weight were observed

[ADDIN EN.CITE <EndNote><Cite><Author>Oishi</Author><Year>1980</Year><RecNum>2</RecNum><DisplayText>(Oishi and Hiraga 1980)</DisplayText><record><rec-number>2</rec-number><foreign-keys><key app="EN" db-id="rpv9f5v5cfrzxgesv5bv9sdn0e9dz2vdpzwx" timestamp="0">2</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Oishi, S.</author><author>Hiraga, K.</author></authors></contributors><titles><title>Testicular atrophy induced by phthalic acid esters: Effect on testosterone and zinc concentrations</title><secondary-title>Toxicology and Applied Pharmacology</secondary-title><alt-title>Toxicol Appl Pharmacol</alt-title><short-title>Toxicology and Applied Pharmacology</short-title></titles><pages>35-41</pages><volume>53</volume><number>1</number><dates><year>1980</year></dates><isbn>ISSN 0041-008X
EISSN 1096-0333</isbn><accession-num>7385236</accession-num><label>61572</label><urls><related-urls><url>https://heronet.epa.gov/heronet/index.cfm/reference/download/reference_id/61572C3 -14,31,32,1097,1480,1547,1628,1826,1837,2188,2195,2205,2206,2265,2294,2305,2320</url></related-urls></urls><custom1>true</custom1><electronic-resource-num>10.1016/0041-008x(80)90378-6</electronic-resource-num><language>English</language></record></Cite></EndNote>]. Given the consistent reductions in offspring postnatal growth across all multigenerational studies including the *high confidence* studies by [HYPERLINK \l "_ENREF_10" \o "Fujii, 2005 #22"] and [HYPERLINK \l "_ENREF_54" \o "RTI International, 1984 #28"], there is *robust* evidence that DEP exposure can affect postnatal growth.

The two *high confidence* studies that examined the incidence of fetal external, skeletal, and visceral malformations in rats [ADDIN EN.CITE <EndNote><Cite><Author>NTP</Author><Year>1988</Year><RecNum>29</RecNum><DisplayText>(NTP 1988)</DisplayText><record><rec-number>29</rec-number><foreign-keys><key app="EN" db-id="rpv9f5v5cfrzxgesv5bv9sdn0e9dz2vdpzwx" timestamp="0">29</key></foreign-keys><ref-type name="Report">27</ref-type><contributors><authors><author>NTP,</author></authors></contributors><titles><title>Developmental toxicity evaluation of diethyl phthalate (CAS No. 84-66-2) administered to CD rats on gestational days 6 through 15</title></titles><dates><year>1988</year></dates><pub-location>Research Triangle Park, NC
</pub-location><isbn>NTP-88-336; RTI-207</isbn><label>1313353</label><urls><related-urls><url>https://heronet.epa.gov/heronet/index.cfm/reference/download/reference_id/1313353C3 -1097,2305</url></related-urls></urls><language>English</language><modified-date>National Toxicology Program</modified-date></record></Cite></EndNote>] and rabbits [ADDIN EN.CITE <EndNote><Cite><Author>Procter & Gamble</Author><Year>1994</Year><RecNum>32</RecNum><DisplayText>(Procter & Gamble 1994)</DisplayText><record><rec-number>32</rec-number><foreign-keys><key app="EN" db-id="rpv9f5v5cfrzxgesv5bv9sdn0e9dz2vdpzwx" timestamp="0">32</key></foreign-keys><ref-type name="Report">27</ref-type><contributors><authors><author>Procter & Gamble,</author></authors></contributors><titles><title>Teratogenicity study of E-2426.01 (diethyl phthalate) by dermal application to rabbits with cover letter dated 05/02/94</title></titles><dates><year>1994</year></dates><pub-location>Washington, DC</pub-location><publisher>U.S. Environmental Protection Agency</publisher><isbn>8EHQ-86940000362</isbn><label>1315778</label><urls><related-

urls><url>https://heronet.epa.gov/heronet/index.cfm/reference/download/reference_id/1315778C3 - 1097,2305</url></related-urls></urls><language>English</language><modified-date>Proctor & Gamble Company</modified-date></record></Cite></EndNote>] found little to no effects of DEP exposure during gestation. However, the study in rats by [HYPERLINK \l "_ENREF_34" \o "NTP, 1988 #29"] observed a dose-related statistically significant increase in the mean percent of fetuses with a rudimentary or full extra (supernumerary) lumbar rib, which is a skeletal variation [ADDIN EN.CITE <EndNote><Cite><Author>US EPA</Author><Year>1991</Year><RecNum>65</RecNum><DisplayText>(US EPA 1991)</DisplayText><record><rec-number>65</rec-number><foreign-keys><key app="EN" db-id="rpv9f5v5cfrzxgesv5bv9sdn0e9dz2vdpzwx" timestamp="0">65</key></foreign-keys><ref-type name="Government Document">46</ref-type><contributors><authors><author>US EPA,</author></authors><secondary-authors><author>Risk Assessment Forum</author></secondary-authors></contributors><titles><title>Guidelines for developmental toxicity risk assessment.</title></titles><dates><year>1991</year></dates><pub-location>Washington DC</pub-location><publisher>U.S. Environmental Protecion Agency</publisher><isbn>EPA/600/FR-91/001</isbn><urls></urls></record></Cite></EndNote>]. The study by [HYPERLINK \l "_ENREF_49" \o "Procter & Gamble, 1994 #32"] in rabbits observed two malformed fetuses in two different litters in the highest DEP dose group (out of 80 total fetuses and 12 total litters in this dose group) and no malformed fetuses in the controls or lower DEP dose groups (out of 77-91 total fetuses and 12 litters per group); the observed malformations consisted of one fetus with fused and split ribs and missing lumbar and coccygeal vertebrae, and one with acrania, hernia umbilicalis, and incurved ribs. The authors did not consider this finding to be treatment-related because the malformations were of different types and the incidence was within the rate of historical controls, although historical control data were not provided as part of this study. There were no dose-related effects on skeletal variations in the rabbit fetuses, including on the number of ribs. Altogether, the evidence for DEP effects on fetal morphological development is considered *slight*.

3.5.2. Synthesis of results for developmental effects

Overall, there is *moderate* evidence that DEP exposure can cause developmental toxicity (Table 4). In particular, decreased postnatal growth in rats and mice was observed in almost all gestational and early postnatal exposure studies that assessed this endpoint, including two studies that were *high confidence* for this endpoint [ADDIN EN.CITE ADDIN EN.CITE.DATA]. Otherwise, effects on survival, growth, and structural alterations were generally not observed. It is notable that in many cases, the observed developmental effects occurred at doses that were also associated with maternal effects (Section 3.5.1). For instance, fetal skeletal variations occurred at a dose concurrent with decreased maternal body weight gain [ADDIN EN.CITE <EndNote><Cite><Author>NTP</Author><Year>1988</Year><RecNum>29</RecNum><DisplayText>(NTP 1988)</DisplayText><record><rec-number>29</rec-number><foreign-keys><key app="EN" db-id="rpv9f5v5cfrzxgesv5bv9sdn0e9dz2vdpzwx" timestamp="0">29</key></foreign-keys><ref-type name="Report">27</ref-type><contributors><authors><author>NTP,</author></authors></contributors><titles><title>Developmental toxicity evaluation of diethyl phthalate (CAS No. 84-66-2) administered to CD rats on gestational days 6 through 15</title></titles><dates><year>1988</year></dates><pub-location>Research Triangle Park, NC
</pub-location><isbn>NTP-88-336; RTI-207</isbn><label>1313353</label><urls><related-

urls><url>https://heronet.epa.gov/heronet/index.cfm/reference/download/reference_id/1313353C3 - 1097,2305</url></related-urls></urls><language>English</language><modified-date>National Toxicology Program</modified-date></record></Cite></EndNote>], and decreased offspring postnatal growth occurred at doses associated with decreased litter size in [ADDIN EN.CITE <EndNote><Cite><Author>RTI International</Author><Year>1984</Year><RecNum>28</RecNum><DisplayText>(RTI International 1984)</DisplayText><record><rec-number>28</rec-number><foreign-keys><key app="EN" db-id="rpv9f5v5cfrzxgesv5bv9sdn0e9dz2vdpzwx" timestamp="0">28</key></foreign-keys><ref-type name="Report">27</ref-type><contributors><authors><author>RTI International,</author></authors><tertiary-authors><author>National Toxicology Program</author></tertiary-authors></contributors><titles><title>Diethyl phthalate: Reproduction and fertility assessment in CD-1 mice when administered in the feed</title></titles><dates><year>1984</year></dates><pub-location>Research Triangle Park, NC</pub-location><publisher><style face="normal" font="default" size="10">RTI International</style></publisher><label>1313352</label><urls><related-urls><url>https://heronet.epa.gov/heronet/index.cfm/reference/download/reference_id/1313352C3 - 1097,2305</url></related-urls></urls><language>English</language></record></Cite></EndNote>] and increased maternal weight gain [ADDIN EN.CITE <EndNote><Cite><Author>Fujii</Author><Year>2005</Year><RecNum>22</RecNum><DisplayText>(Fujii et al. 2005)</DisplayText><record><rec-number>22</rec-number><foreign-keys><key app="EN" db-id="rpv9f5v5cfrzxgesv5bv9sdn0e9dz2vdpzwx" timestamp="0">22</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Fujii, S.</author><author>Yabe, K.</author><author>Furukawa, M.</author><author>Hirata, M.</author><author>Kiguchi, M.</author><author>Ikka, T.</author></authors></contributors><titles><title>A two-generation reproductive toxicity study of diethyl phthalate (DEP) in rats</title><secondary-title>Journal of Toxicological Sciences</secondary-title><alt-title>J Toxicol Sci</alt-title><short-title>Journal of Toxicological Sciences</short-title></titles><pages>S97-116</pages><volume>30</volume><number>Special Issue P</number><dates><year>2005</year></dates><isbn>ISSN 0388-1350
EISSN 1880-3989</isbn><accession-num>16641546</accession-num><label>1298274</label><urls><related-urls><url>https://heronet.epa.gov/heronet/index.cfm/reference/download/reference_id/1298274C3 - 1097,2187,2212,2245,2294,2305,2344</url></related-urls></urls><custom1>true</custom1><electronic-resource-num>10.2131/jts.30.S97</electronic-resource-num><language>English</language></record></Cite></EndNote>]. This indicates that DEP is not a potent developmental toxicant, even though multiple developmental effects were observed.

Although the number of studies was limited, the available body of literature for developmental DEP exposure includes a variety of experimental designs covering exposure during critical windows of fetal and postnatal development, including two studies that examined outcomes in offspring following exposure over two generations [ADDIN EN.CITE ADDIN EN.CITE.DATA]. Another strength is that several species were examined in these studies.

3.6. Liver effects

A figure indicating the doses at which statistically significant effects on liver occurred is provided in the Supplementary Materials (Figure S13 -S15 and S18).

3.6.1. *Summary of available studies*

Increases in liver weight in rats and mice were reported in multiple *high* and *medium confidence* studies that tested oral and dermal exposures of DEP. Statistically significant increases in relative liver weight ranged from 7 to 33%, and were generally observed at the highest dose tested (>1000 mg/kg-day) [ADDIN EN.CITE ADDIN EN.CITE.DATA]. In a 2-year dermal exposure study, relative liver weights were statistically significantly increased following 4 weeks of exposure in rats and mice, but not at the 15-month evaluation [ADDIN EN.CITE <EndNote><Cite><Author>NTP</Author><Year>1995</Year><RecNum>27</RecNum><DisplayText>(NTP 1995)</DisplayText><record><rec-number>27</rec-number><foreign-keys><key app="EN" db-id="rpv9f5v5cfrzxgesv5bv9sdn0e9dz2vdpzwx" timestamp="0">27</key></foreign-keys><ref-type name="Report">27</ref-type><contributors><authors><author>NTP,</author></authors></contributors><titles><title>Toxicology and carcinogenesis studies of diethylphthalate (CAS No. 84-66-2) in F344/n rats and B6C3F1 mice (dermal studies) with dermal initiation/ promotion study of diethylphthalate and dimethylphthalate (CAS No. 131-11-3) in male Swiss (cd-1(r)) mice</title></titles><pages>1-286</pages><volume>429</volume><dates><year>1995</year></dates><pub-location>Research Triangle Park, NC</pub-location><isbn>NTP TR 429</isbn><accession-num>12616302</accession-num><label>1313305</label><work-type>NTP</work-type><urls><related-urls><url>https://heronet.epa.gov/heronet/index.cfm/reference/download/reference_id/1313305C3 -1097,2245,2305,2366</url></related-urls></urls><language>English</language><modified-date>National Toxicology Program</modified-date></record></Cite></EndNote>]. One potential mechanism for the increase in liver weight may be the induction of peroxisome proliferation. [HYPERLINK \l "_ENREF_10" \o "Fujii, 2005 #22"] reported a statistically significant increase at > 1150 mg/kg-day in the microsomal Cyp4A1 isoenzyme induced in rodents by peroxisome proliferators. Peroxisome proliferation was only slightly increased in rat hepatocytes following exposure to DEP despite a statistically significant increase in peroxisomal associated enzyme carnitine acetyltransferase and relative liver weight at 1753 mg/kg-day [ADDIN EN.CITE <EndNote><Cite><Author>Moody</Author><Year>1978</Year><RecNum>16</RecNum><DisplayText>(Moody and Reddy 1978)</DisplayText><record><rec-number>16</rec-number><foreign-keys><key app="EN" db-id="rpv9f5v5cfrzxgesv5bv9sdn0e9dz2vdpzwx" timestamp="0">16</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Moody, D.</author><author>Reddy, J.</author></authors></contributors><titles><title>Hepatic peroxisome (microbody) proliferation in rats fed plasticizers and related compounds</title><secondary-title>Toxicology and Applied Pharmacology</secondary-title><alt-title>Toxicol Appl Pharmacol</alt-title><short-title>Toxicology and Applied Pharmacology</short-title></titles><pages>497-504</pages><volume>45</volume><number>2</number><dates><year>1978</year></dates><isbn>ISSN 0041-008X
EISSN 1096-0333</isbn><accession-num>705785</accession-num><label>698561</label><urls><related-urls><url>https://heronet.epa.gov/heronet/index.cfm/reference/download/reference_id/698561C3 -1097,1547,1826,2042,2205,2265,2305,2485</url></related-urls></urls><custom1>true</custom1><electronic-resource-num>10.1016/0041-008x(78)90111-4</electronic-resource-num><language>English</language></record></Cite></EndNote>]. In contrast, in rats, there was no effect on absolute liver weight following gestational exposure in F1 male rats at 750 mg/kg-day DEP [ADDIN EN.CITE

<EndNote><Cite><Author>Gray</Author><Year>2000</Year><RecNum>10</RecNum><DisplayText>(Gray et al. 2000)</DisplayText><record><rec-number>10</rec-number><foreign-keys><key app="EN" db-id="rpv9f5v5cfrzxgesv5bv9sdn0e9dz2vdpzwx" timestamp="0">10</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Gray, L. E., Jr</author><author>Ostby, J.</author><author>Furr, J.</author><author>Price, M.</author><author>Veeramachaneni, D. N. R.</author><author>Parks, L.</author></authors></contributors><titles><title>Perinatal exposure to the phthalates DEHP, BBP, and DNIP, but not DEP, DMP, or DOTP, alters sexual differentiation of the male rat</title><secondary-title>Toxicological Sciences</secondary-title><alt-title>Toxicol Sci</alt-title><short-title>Toxicological Sciences</short-title></titles><periodical><full-title>Toxicological Sciences</full-title><abbr-1>Toxicol Sci</abbr-1></periodical><alt-periodical><full-title>Toxicological Sciences</full-title><abbr-1>Toxicol Sci</abbr-1></alt-periodical><pages>350-365</pages><volume>58</volume><number>2</number><dates><year>2000</year></dates><isbn>ISSN 1096-6080
EISSN 1096-0929</isbn><accession-num>11099647</accession-num><label>678742</label><urls><related-urls><url>https://heronet.epa.gov/heronet/index.cfm/reference/download/reference_id/678742C3 - 1097,1547,1826,2205,2206,2207,2212,2245,2247,2294,2305,2366,2370,2424,2516,2642</url></related-urls></urls><custom1>true</custom1><electronic-resource-num>10.1093/toxsci/58.2.350</electronic-resource-num><language>English</language></record></Cite></EndNote>] and no effect on relative liver weight in adult animals following 2 or 4-week exposures to 500 mg/kg-day DEP or 250 mg/kg-day MEP [ADDIN EN.CITE ADDIN EN.CITE.DATA] or up to 1000 mg/kg-day DEP [ADDIN EN.CITE <EndNote><Cite><Author>Shiraishi</Author><Year>2006</Year><RecNum>30</RecNum><DisplayText>(Shiraishi et al. 2006)</DisplayText><record><rec-number>30</rec-number><foreign-keys><key app="EN" db-id="rpv9f5v5cfrzxgesv5bv9sdn0e9dz2vdpzwx" timestamp="0">30</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Shiraishi, K.</author><author>Miyata, K.</author><author>Houshuyama, S.</author><author>Imatanaka, N.</author><author>Umano, T.</author><author>Minobe, Y.</author><author>Yamasaki, K.</author></authors></contributors><titles><title>Subacute oral toxicity study of diethylphthalate based on the draft protocol for "Enhanced OECD Test Guideline no. 407&quot</title><secondary-title>Archives of Toxicology</secondary-title><alt-title>Arch Toxicol</alt-title><short-title>Archives of Toxicology</short-title></titles><pages>10-16</pages><volume>80</volume><number>1</number><dates><year>2006</year></dates><isbn>ISSN 0340-5761
EISSN 1432-0738</isbn><accession-num>16059724</accession-num><label>1315363</label><urls><related-urls><url>https://heronet.epa.gov/heronet/index.cfm/reference/download/reference_id/1315363C3 - 1097,2204,2212,2305,2516</url></related-urls></urls><custom1>true</custom1><electronic-resource-num>10.1007/s00204-005-0008-6</electronic-resource-num><language>English</language></record></Cite></EndNote>]. The lack of effect on organ weight may be due to differences in the exposure window and/or dose. In a series of low dose (0.57-6.25 mg/kg-day) studies, effects on liver weights were inconsistent, with studies reporting increases, decreases, or no effect on liver weight following oral DEP exposures from 90 to 150 days [ADDIN EN.CITE ADDIN EN.CITE.DATA]. These low dose studies were considered *low confidence* due to the concerns discussed in Section 3.2. Despite these inconsistencies, increased liver weights were observed

across multiple studies, and the evidence for changes in liver weight following DEP exposure is considered *moderate*.

Data on histopathological changes differed across studies. In three *medium* or *high confidence* oral repeat-dose studies, dose-related histopathological changes in the liver were not observed in rats of either sex following 42 or 112 days of exposure to doses up to 3,160 mg/kg-day [ADDIN EN.CITE <EndNote><Cite><Author>Brown</Author><Year>1978</Year><RecNum>72</RecNum><DisplayText>(Brown et al. 1978)</DisplayText><record><rec-number>72</rec-number><foreign-keys><key app="EN" db-id="rpv9f5v5cfrzxgesv5bv9sdn0e9dz2vdpzwx" timestamp="0">72</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Brown, D.</author><author>Butterworth, K. R.</author><author>Gaunt, I. F.</author><author>Grasso, P.</author><author>Gangolli, S. D.</author></authors></contributors><titles><title>Short-term oral toxicity study of diethyl phthalate in the rat</title><secondary-title>Food and Cosmetics Toxicology</secondary-title><alt-title>Food Cosmet Toxicol</alt-title></titles><pages>415-422</pages><volume>16</volume><number>5</number><dates><year>1978</year></dates><isbn>ISSN 0015-6264
EISSN 1878-6049</isbn><accession-num>711065</accession-num><label>1313235</label><urls><related-urls><url>http://dx.doi.org/10.1016/S0015-6264(78)80258-2</url></related-urls></urls><electronic-resource-num>10.1016/S0015-6264(78)80258-2</electronic-resource-num><language>English</language></record></Cite></EndNote>] or 4 weeks of exposure to doses up to 1000 mg/kg-day [HYPERLINK \l "_ENREF_57" \o "Shiraishi, 2006 #30"], or in F0 and F1 rats in a multigenerational reproductive study at doses up to 1,016 to 1,297 mg/kg-day (F0) and 1,150 to 1,375 mg/kg-day (F1) [ADDIN EN.CITE <EndNote><Cite><Author>Fujii</Author><Year>2005</Year><RecNum>22</RecNum><DisplayText>(Fujii et al. 2005)</DisplayText><record><rec-number>22</rec-number><foreign-keys><key app="EN" db-id="rpv9f5v5cfrzxgesv5bv9sdn0e9dz2vdpzwx" timestamp="0">22</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Fujii, S.</author><author>Yabe, K.</author><author>Furukawa, M.</author><author>Hirata, M.</author><author>Kiguchi, M.</author><author>Ikka, T.</author></authors></contributors><titles><title>A two-generation reproductive toxicity study of diethyl phthalate (DEP) in rats</title><secondary-title>Journal of Toxicological Sciences</secondary-title><alt-title>J Toxicol Sci</alt-title><short-title>Journal of Toxicological Sciences</short-title></titles><pages>S97-116</pages><volume>30</volume><number>Special Issue P</number><dates><year>2005</year></dates><isbn>ISSN 0388-1350
EISSN 1880-3989</isbn><accession-num>16641546</accession-num><label>1298274</label><urls><related-urls><url>https://heronet.epa.gov/heronet/index.cfm/reference/download/reference_id/1298274C3 -1097,2187,2212,2245,2294,2305,2344</url></related-urls></urls><custom1>true</custom1><electronic-resource-num>10.2131/jts.30.S97</electronic-resource-num><language>English</language></record></Cite></EndNote>]. Similarly, except for a statistically significant increased incidence of basophilic foci in the liver of male mice at 16.8 mg/kg-day, no dose-related histopathological changes in liver were reported in the *high confidence* two-year dermal exposure study in mice at doses up to 33.6 mg/kg-day [ADDIN EN.CITE <EndNote><Cite><Author>NTP</Author><Year>1995</Year><RecNum>27</RecNum><DisplayText>(NTP 1995)</DisplayText><record><rec-number>27</rec-number><foreign-keys><key app="EN" db-id="rpv9f5v5cfrzxgesv5bv9sdn0e9dz2vdpzwx" timestamp="0">27</key></foreign-keys><ref-type name="Report">27</ref-

type><contributors><authors><author>NTP,</author></authors></contributors><titles><title>Toxicology and carcinogenesis studies of diethylphthalate (CAS No. 84-66-2) in F344/n rats and B6C3F1 mice (dermal studies) with dermal initiation/ promotion study of diethylphthalate and dimethylphthalate (CAS No. 131-11-3) in male Swiss (cd-1(r)) mice</title></titles><pages>1-286</pages><volume>429</volume><dates><year>1995</year></dates><pub-location>Research Triangle Park, NC</pub-location><isbn>NTP TR 429</isbn><accession-num>12616302</accession-num><label>1313305</label><work-type>NTP</work-type><urls><related-urls><url>https://heronet.epa.gov/heronet/index.cfm/reference/download/reference_id/1313305C3 - 1097,2245,2305,2366</url></related-urls></urls><language>English</language><modified-date>National Toxicology Program</modified-date></record></Cite></EndNote>]. Conversely, histopathological changes including vacuolization, lipid droplets in the liver, loss of hepatic architecture and necrotic changes were reported in the series of low dose (0.57 to 6.25 mg/kg-day) studies considered *low confidence* due to the concerns discussed in Section 3.2 [ADDIN EN.CITE ADDIN EN.CITE.DATA]the histopathological reports from this group did not include quantitative data, which contributed to the low confidence in these findings. Given the lack of confidence in the low dose findings along with the negative findings at higher doses over various periods of exposure, the evidence for histopathological effects is considered *indeterminate*.

Four *high confidence* studies reported limited evidence of effects on biochemical markers of hepatocellular or hepatobiliary liver toxicity following DEP exposure. Statistically significantly decreased aspartate aminotransferase (AST) activity, a non-specific marker of liver injury, was reported in male rats following exposure to up to 1000 mg/kg-day for 28 days by [ADDIN EN.CITE <EndNote><Cite><Author>Shiraishi</Author><Year>2006</Year><RecNum>30</RecNum><DisplayText>(Shiraishi et al. 2006)</DisplayText><record><rec-number>30</rec-number><foreign-keys><key app="EN" db-id="rpv9f5v5cfrzxgesv5bv9sdn0e9dz2vdpzwx" timestamp="0">30</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Shiraishi, K.</author><author>Miyata, K.</author><author>Houshuyama, S.</author><author>Imatanaka, N.</author><author>Umano, T.</author><author>Minobe, Y.</author><author>Yamasaki, K.</author></authors></contributors><titles><title>Subacute oral toxicity study of diethylphthalate based on the draft protocol for "Enhanced OECD Test Guideline no. 407&quot</title><secondary-title>Archives of Toxicology</secondary-title><alt-title>Arch Toxicol</alt-title><short-title>Archives of Toxicology</short-title></titles><pages>10-16</pages><volume>80</volume><number>1</number><dates><year>2006</year></dates><isbn>ISSN 0340-5761
EISSN 1432-0738</isbn><accession-num>16059724</accession-num><label>1315363</label><urls><related-urls><url>https://heronet.epa.gov/heronet/index.cfm/reference/download/reference_id/1315363C3 - 1097,2204,2212,2305,2516</url></related-urls></urls><custom1>true</custom1><electronic-resource-num>10.1007/s00204-005-0008-6</electronic-resource-num><language>English</language></record></Cite></EndNote>]. The biological significance of decreased AST activity is unclear. Findings in rats exposed to 500 mg/kg-day DEP or 250 mg/kg-day MEP for 2 or 4 weeks showed only minimal (0-21%) non-statistically significant increases in alanine aminotransferase (ALT), a liver specific marker, or AST activity in serum compared to controls [ADDIN EN.CITE ADDIN EN.CITE.DATA]. Statistically significant increases in the hepatobiliary marker, gamma-glutamyl transferase (GGT), were reported at 40 and 1000 mg/kg-day in male Sprague-Dawley rats [ADDIN EN.CITE

<EndNote><Cite><Author>Shiraishi</Author><Year>2006</Year><RecNum>30</RecNum><DisplayText>(Shiraishi et al. 2006)</DisplayText><record><rec-number>30</rec-number><foreign-keys><key app="EN" db-id="rpv9f5v5cfrzxgesv5bv9sdn0e9dz2vdpzwx" timestamp="0">30</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Shiraishi, K.</author><author>Miyata, K.</author><author>Houshuyama, S.</author><author>Imatanaka, N.</author><author>Umano, T.</author><author>Minobe, Y.</author><author>Yamasaki, K.</author></authors></contributors><titles><title>Subacute oral toxicity study of diethylphthalate based on the draft protocol for "Enhanced OECD Test Guideline no. 407&quot</title><secondary-title>Archives of Toxicology</secondary-title><alt-title>Arch Toxicol</alt-title><short-title>Archives of Toxicology</short-title></titles><pages>10-16</pages><volume>80</volume><number>1</number><dates><year>2006</year></dates><isbn>ISSN 0340-5761
EISSN 1432-0738</isbn><accession-num>16059724</accession-num><label>1315363</label><urls><related-urls><url>https://heronet.epa.gov/heronet/index.cfm/reference/download/reference_id/1315363C3 - 1097,2204,2212,2305,2516</url></related-urls></urls><custom1>true</custom1><electronic-resource-num>10.1007/s00204-005-0008-6</electronic-resource-num><language>English</language></record></Cite></EndNote>] Additionally, a statistically significant increase in alkaline phosphatase (ALP) activity, also marker of hepatobiliary injury, was observed in female rats following a 15-month dermal exposure, with no effects observed in male rats [ADDIN EN.CITE <EndNote><Cite><Author>NTP</Author><Year>1995</Year><RecNum>27</RecNum><DisplayText>(NTP 1995)</DisplayText><record><rec-number>27</rec-number><foreign-keys><key app="EN" db-id="rpv9f5v5cfrzxgesv5bv9sdn0e9dz2vdpzwx" timestamp="0">27</key></foreign-keys><ref-type name="Report">27</ref-type><contributors><authors><author>NTP,</author></authors></contributors><titles><title>Toxicology and carcinogenesis studies of diethylphthalate (CAS No. 84-66-2) in F344/n rats and B6C3F1 mice (dermal studies) with dermal initiation/ promotion study of diethylphthalate and dimethylphthalate (CAS No. 131-11-3) in male Swiss (cd-1(r)) mice</title></titles><pages>1-286</pages><volume>429</volume><dates><year>1995</year></dates><pub-location>Research Triangle Park, NC</pub-location><isbn>NTP TR 429</isbn><accession-num>12616302</accession-num><label>1313305</label><work-type>NTP</work-type><urls><related-urls><url>https://heronet.epa.gov/heronet/index.cfm/reference/download/reference_id/1313305C3 - 1097,2245,2305,2366</url></related-urls></urls><language>English</language><modified-date>National Toxicology Program</modified-date></record></Cite></EndNote>]. In contrast, statistically significant changes in the serum levels of ALT, AST, ALP, sorbitol dehydrogenase (SDH), lactate dehydrogenase (LDH), and were frequently reported in rats and mice in the series low dose studies considered *low confidence* due to the concerns discussed in Section 3.2 [ADDIN EN.CITE ADDIN EN.CITE.DATA]. Changes in enzyme levels were observed in both sexes and across generations; however, the number of animals utilized to assess enzymes changes was typically small ($n \leq 6$) and the findings were not always consistent. For example, one low dose study from this group reported decreased serum LDH activity levels in male and female rats without changes in serum levels of AST, SDH or ALP (Sinkar and Rao 2007). Other reported statistically significant changes in clinical chemistry following low dose exposures were increased serum triglycerides, serum glucose and liver glycogen levels in mice and rats [ADDIN EN.CITE ADDIN EN.CITE.DATA], increased cholesterol in rats [ADDIN

EN.CITE ADDIN EN.CITE.DATA], and increases in measurements of lipid peroxidation and decreased glutathione and glutathione reductase levels [ADDIN EN.CITE ADDIN EN.CITE.DATA]. In one *high confidence* study, a statistically significant increase in the fatty acid oxidizing enzyme CYP4A was reported at ≥ 1150 mg/kg-day, supportive of alterations in lipid metabolism [ADDIN EN.CITE <EndNote><Cite><Author>Fujii</Author><Year>2005</Year><RecNum>22</RecNum><DisplayText>(Fujii et al. 2005)</DisplayText><record><rec-number>22</rec-number><foreign-keys><key app="EN" db-id="rpv9f5v5cfrzxgesv5bv9sdn0e9dz2vdpzwx" timestamp="0">22</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Fujii, S.</author><author>Yabe, K.</author><author>Furukawa, M.</author><author>Hirata, M.</author><author>Kiguchi, M.</author><author>Ikka, T.</author></authors></contributors><titles><title>A two-generation reproductive toxicity study of diethyl phthalate (DEP) in rats</title><secondary-title>Journal of Toxicological Sciences</secondary-title><alt-title>J Toxicol Sci</alt-title><short-title>Journal of Toxicological Sciences</short-title></titles><pages>S97-116</pages><volume>30</volume><number>Special Issue P</number><dates><year>2005</year></dates><isbn>ISSN 0388-1350
EISSN 1880-3989</isbn><accession-num>16641546</accession-num><label>1298274</label><urls><related-urls><url>https://heronet.epa.gov/heronet/index.cfm/reference/download/reference_id/1298274C3 -1097,2187,2212,2245,2294,2305,2344</url></related-urls></urls><custom1>true</custom1><electronic-resource-num>10.2131/jts.30.S97</electronic-resource-num><language>English</language></record></Cite></EndNote>]. In addition, in one medium confidence study the activity of two peroxisome associated enzymes, catalase and carnitine acetyltransferase, was statistically increased in male rats following subchronic exposure [ADDIN EN.CITE <EndNote><Cite><Author>Moody</Author><Year>1978</Year><RecNum>16</RecNum><DisplayText>(Moody and Reddy 1978)</DisplayText><record><rec-number>16</rec-number><foreign-keys><key app="EN" db-id="rpv9f5v5cfrzxgesv5bv9sdn0e9dz2vdpzwx" timestamp="0">16</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Moody, D.</author><author>Reddy, J.</author></authors></contributors><titles><title>Hepatic peroxisome (microbody) proliferation in rats fed plasticizers and related compounds</title><secondary-title>Toxicology and Applied Pharmacology</secondary-title><alt-title>Toxicol Appl Pharmacol</alt-title><short-title>Toxicology and Applied Pharmacology</short-title></titles><pages>497-504</pages><volume>45</volume><number>2</number><dates><year>1978</year></dates><isbn>ISSN 0041-008X
EISSN 1096-0333</isbn><accession-num>705785</accession-num><label>698561</label><urls><related-urls><url>https://heronet.epa.gov/heronet/index.cfm/reference/download/reference_id/698561C3 -1097,1547,1826,2042,2205,2265,2305,2485</url></related-urls></urls><custom1>true</custom1><electronic-resource-num>10.1016/0041-008x(78)90111-4</electronic-resource-num><language>English</language></record></Cite></EndNote>]. Overall, statistically significant increases in liver enzyme activity indicative of liver toxicity was observed mostly in *low confidence* studies except for increased ALP activity in female rats following dermal exposure; therefore, the evidence for effects on biochemical markers of liver toxicity is *slight*.

3.6.2. Synthesis of results for liver effects

The available toxicology studies in rodents provide *moderate* evidence of liver toxicity following DEP exposure (Table 5). Evidence for liver toxicity in experimental animal studies includes reports on liver weight and clinical chemistry, as well as histopathology findings. Statistically significant increases in liver weight were demonstrated in several *high*, *medium*, and *low confidence* studies of rats and mice across multiple routes of exposure, with effects observed more consistently at higher doses in *high* and *medium confidence* studies [ADDIN EN.CITE ADDIN EN.CITE.DATA]. Histopathological and biochemical changes were frequently reported in a series of *low confidence*, low-dose studies of DEP in rats and mice whereas there was little evidence of significant histopathological or biochemical changes in *high* and *medium* confidence studies. Effects of DEP on liver weight may be due to its actions as a peroxisome proliferator, which is a common mechanism among phthalates; however, DEP is considered a relatively weak peroxisome proliferator [ADDIN EN.CITE ADDIN EN.CITE.DATA]. Therefore, other adaptive mechanisms may play a role in changes in liver weight at higher exposures of DEP [ADDIN EN.CITE ADDIN EN.CITE.DATA].

Strengths of the evidence base for hepatic effects include the availability of a variety of experimental designs covering a range of doses. The most remarkable findings were observed in low dose studies that were considered *low confidence*. Therefore, the database for liver effects would be strengthened by additional research at exposures in the lower dose range using more robust experimental designs.

3.7. Kidney effects

A figure indicating the doses at which statistically significant effects on kidney weight occurred is provided in the Supplementary Materials (Figure S16).

3.7.1. Summary of available studies

Changes in kidney weight following oral DEP exposure in rats and mice were inconsistent. Multiple *high* and *medium confidence* oral exposure studies in rats reported statistically significant increases or decreases in relative and/or absolute kidney weight, with no clear or consistent pattern of effect across studies [ADDIN EN.CITE ADDIN EN.CITE.DATA]. One *high confidence* dermal study in rats and mice reported a statistically significant increase in relative kidney weight in female mice after 15 months of exposure; however, in male mice, absolute kidney weight was statistically significantly decreased over the same exposure period, and there were no effects on absolute or relative kidney weight in male or female rats in this study [ADDIN EN.CITE <EndNote><Cite><Author>NTP</Author><Year>1995</Year><RecNum>27</RecNum><DisplayText>(NTP 1995)</DisplayText><record><rec-number>27</rec-number><foreign-keys><key app="EN" db-id="rpv9f5v5cfrzxgesv5bv9sdn0e9dz2vdpzwx" timestamp="0">27</key></foreign-keys><ref-type name="Report">27</ref-type><contributors><authors><author>NTP,</author></authors></contributors><titles><title>Toxicology and carcinogenesis studies of diethylphthalate (CAS No. 84-66-2) in F344/n rats and B6C3F1 mice (dermal studies) with dermal initiation/ promotion study of diethylphthalate and dimethylphthalate (CAS No. 131-11-3) in male Swiss (cd-1(r)) mice</title></titles><pages>1-286</pages><volume>429</volume><dates><year>1995</year></dates><pub-location>Research Triangle Park, NC</pub-location><isbn>NTP TR 429</isbn><accession-num>12616302</accession-num><label>1313305</label><work-type>NTP</work-type><urls><related-urls><url>https://heronet.epa.gov/heronet/index.cfm/reference/download/reference_id/1313305C3 -1097,2245,2305,2366</url></related-urls></urls><language>English</language><modified-date>National Toxicology Program</modified-date></record></Cite></EndNote>]. Dose-related increases or decreases in kidney weight were not observed in rats in three *high* or *medium confidence* peripubertal exposure studies [ADDIN EN.CITE ADDIN EN.CITE.DATA] or in the *high confidence* study that exposed rats from GD 14 – PND 3 [ADDIN EN.CITE <EndNote><Cite><Author>Gray</Author><Year>2000</Year><RecNum>10</RecNum><DisplayText>(Gray et al. 2000)</DisplayText><record><rec-number>10</rec-number><foreign-keys><key app="EN" db-id="rpv9f5v5cfrzxgesv5bv9sdn0e9dz2vdpzwx" timestamp="0">10</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Gray, L. E., Jr</author><author>Ostby, J.</author><author>Furr, J.</author><author>Price, M.</author><author>Veeramachaneni, D. N. R.</author><author>Parks, L.</author></authors></contributors><titles><title>Perinatal exposure to the phthalates DEHP, BBP, and DNIP, but not DEP, DMP, or DOTP, alters sexual differentiation of the male rat</title><secondary-title>Toxicological Sciences</secondary-title><alt-title>Toxicol Sci</alt-title><short-title>Toxicological Sciences</short-title></titles><periodical><full-title>Toxicological Sciences</full-title><abbr-1>Toxicol Sci</abbr-1></periodical><alt-periodical><full-title>Toxicological Sciences</full-title><abbr-1>Toxicol Sci</abbr-1></alt-periodical><pages>350-365</pages><volume>58</volume><number>2</number><dates><year>2000</year></dates><isbn>ISSN 1096-6080
EISSN 1096-0929</isbn><accession-num>11099647</accession-num><label>678742</label><urls><related-urls><url>https://heronet.epa.gov/heronet/index.cfm/reference/download/reference_id/678742C3 -1097,1547,1826,2205,2206,2207,2212,2245,2247,2294,2305,2366,2370,2424,2516,2642</url></related-urls></urls><custom1>true</custom1><electronic-resource-num>10.1093/toxsci/58.2.350</electronic-resource-num><language>English</language></record></Cite></EndNote>]. Taken together, because of inconsistent changes in kidney weight in mice and rats, the evidence for effects on kidney weight is considered *indeterminate*

Histopathological analyses of the kidney were conducted in four of the same studies that evaluated kidney weight in rats or mice [ADDIN EN.CITE ADDIN EN.CITE.DATA]. In all cases, no dose-related histopathological changes of the kidney were reported. Altogether, given the lack of effect across studies, the evidence for histopathological effects in the kidney is considered *indeterminate*.

A statistically significant increase in serum calcium levels compared to controls was observed in rats exposed to 500 mg/kg-day for 2 or 4 weeks in the studies by [HYPERLINK \l "_ENREF_24" \o "Kwack, 2010 #87"] and [HYPERLINK \l "_ENREF_23" \o "Kwack, 2009 #14"], respectively; whereas, other biochemical parameters related to the kidney were not affected in these animals (urinary protein level and serum creatinine, creatinine kinase, blood urea nitrogen, and albumin). In the study in rats by [HYPERLINK \l "_ENREF_3" \o "Brown, 1978 #72"], effects on urinary cell excretion were inconsistent, with a statistically significant decrease observed in males after 13 weeks of exposure to 3,160 mg/kg-day but otherwise there were no dose or time-related trends. Since the only consistent effect was increased serum calcium levels, the evidence for effects on biochemical markers of kidney damage is considered *indeterminate*.

3.7.2. Synthesis of results for kidney effects

It was concluded that there was *indeterminate* evidence for kidney toxicity following exposure to DEP (Table 6). The most convincing evidence for kidney toxicity comes from *medium* and *high confidence* studies that observed statistically significant increases or decreases in absolute and/or relative kidney weight at higher doses, although these effects on kidney weight were inconsistent both within and across studies. Minimal data were available on biochemical measurements that are indicative of kidney toxicity. Changes in kidney weight were not supported by histopathological changes in male or female rat kidney.

3.8. Cancer

A 2-year dermal bioassay in rats and mice demonstrated an increased incidence of liver neoplasms in mice following dermal DEP exposure (Figure S17) [ADDIN EN.CITE <EndNote><Cite><Author>NTP</Author><Year>1995</Year><RecNum>27</RecNum><DisplayText>(NTP 1995)</DisplayText><record><rec-number>27</rec-number><foreign-keys><key app="EN" db-id="rpv9f5v5cfrzxgesv5bv9sdn0e9dz2vdpzwx" timestamp="0">27</key></foreign-keys><ref-type name="Report">27</ref-type><contributors><authors><author>NTP,</author></authors></contributors><titles><title>Toxicology and carcinogenesis studies of diethylphthalate (CAS No. 84-66-2) in F344/n rats and B6C3F1 mice (dermal studies) with dermal initiation/ promotion study of diethylphthalate and dimethylphthalate (CAS No. 131-11-3) in male Swiss (cd-1(r)) mice</title></titles><pages>1-286</pages><volume>429</volume><dates><year>1995</year></dates><pub-location>Research Triangle Park, NC</pub-location><isbn>NTP TR 429</isbn><accession-num>12616302</accession-num><label>1313305</label><work-type>NTP</work-type><urls><related-urls><url>https://heronet.epa.gov/heronet/index.cfm/reference/download/reference_id/1313305C3 - 1097,2245,2305,2366</url></related-urls></urls><language>English</language><modified-date>National Toxicology Program</modified-date></record></Cite></EndNote>]. Significant increases in tumor formation in other tissues was not observed in this study. The combined incidence of adenoma or carcinoma was increased in males exposed to 33 mg/day, along with a positive dose-related trend; however, the increase is within NTP's historical control values for 2-year mouse studies. In treated females, combined incidence of adenoma or carcinoma was increased as compared to control but was not related to dose. No evidence of carcinogenic activity of DEP was observed in F344N rats, but sensitivity was reduced due to low survival rates in the rats. In addition, 1-year initiation/promotion studies in CD-1 mice that tested DEP with and without the skin tumor promoter 12-O-tetradecanoylphorbol- 13-acetate (TPA) with or without the skin tumor initiator 7,12-dimethylbenz[a]anthrancene (DMBA) demonstrated that DEP was not able to imitate or promote skin neoplasms [ADDIN EN.CITE <EndNote><Cite><Author>NTP</Author><Year>1995</Year><RecNum>27</RecNum><DisplayText>(NTP 1995)</DisplayText><record><rec-number>27</rec-number><foreign-keys><key app="EN" db-id="rpv9f5v5cfrzxgesv5bv9sdn0e9dz2vdpzwx" timestamp="0">27</key></foreign-keys><ref-type name="Report">27</ref-type><contributors><authors><author>NTP,</author></authors></contributors><titles><title>Toxicology and carcinogenesis studies of diethylphthalate (CAS No. 84-66-2) in F344/n rats and B6C3F1 mice (dermal studies) with dermal initiation/ promotion study of diethylphthalate and dimethylphthalate (CAS No. 131-11-3) in male Swiss (cd-1(r)) mice</title></titles><pages>1-286</pages><volume>429</volume><dates><year>1995</year></dates><pub-location>Research Triangle Park, NC</pub-location><isbn>NTP TR 429</isbn><accession-num>12616302</accession-num><label>1313305</label><work-type>NTP</work-type><urls><related-urls><url>https://heronet.epa.gov/heronet/index.cfm/reference/download/reference_id/1313305C3 - 1097,2245,2305,2366</url></related-urls></urls><language>English</language><modified-date>National Toxicology Program</modified-date></record></Cite></EndNote>]. In the absence of increased tumor incidence above background levels and negative findings in the initiation and promotion studies, the evidence of liver tumorgenicity in experimental animals dermally exposed is considered *indeterminate* (Table 7).

4. DISCUSSION

The results of this systematic review provide *moderate* evidence that DEP causes male reproductive toxicity, developmental toxicity, and liver toxicity, and *slight* evidence for female reproductive toxicity. Evidence for effects on kidney toxicity and cancer was considered *indeterminate*.

There were several outcomes for which no effects were observed, most notably fetal testosterone production and fetal growth. However, in no case was there found to be enough evidence to warrant a conclusion of *compelling evidence of no effect*. A judgement of *compelling evidence of no effect* represents an uncommon situation where no association was identified from extensive evidence across a range of populations and exposures. In the case of fetal testosterone and other androgen-dependent developmental outcomes, all available studies were conducted in the same species (rat) and used mostly similar experimental designs, whereas exposures in multiple species and exposure scenarios would be necessary to rule out the possibility of effect. For fetal growth, studies were available from three species (rats, mice, rabbits) and multiple exposure scenarios (gestation-only and multigenerational studies), but the number of studies (five) was still relatively low.

Although DEP induced some developmental outcomes in rats and mice (decreased postnatal growth, increased supernumerary ribs), there were no effects on fetal growth, and only one study indicated effects on fetal survival [ADDIN EN.CITE <EndNote><Cite><Author>RTI International</Author><Year>1984</Year><RecNum>28</RecNum><DisplayText>(RTI International 1984)</DisplayText><record><rec-number>28</rec-number><foreign-keys><key app="EN" db-id="rpv9f5v5cfrzxgesv5bv9sdn0e9dz2vdpzwx" timestamp="0">28</key></foreign-keys><ref-type name="Report">27</ref-type><contributors><authors><author>RTI International,</author></authors><tertiary-authors><author>National Toxicology Program</author></tertiary-authors></contributors><titles><title>Diethyl phthalate: Reproduction and fertility assessment in CD-1 mice when administered in the feed</title></titles><dates><year>1984</year></dates><pub-location>Research Triangle Park, NC</pub-location><publisher><style face="normal" font="default" size="10">RTI International</style></publisher><label>1313352</label><urls><related-urls><url>https://heronet.epa.gov/heronet/index.cfm/reference/download/reference_id/1313352C3 - 1097,2305</url></related-urls></urls><language>English</language></record></Cite></EndNote>]. Other developmentally toxic phthalates have been found to decrease fetal survival, and it has been hypothesized that phthalate-induced decreases in fetal testosterone production and fetal survival may both be caused by decreased steroidogenesis (decreased testicular testosterone production in male fetuses and decreased ovarian progesterone production in dams) [ADDIN EN.CITE <EndNote><Cite><Author>Howdeshell</Author><Year>2008</Year><RecNum>8</RecNum><DisplayText>(Howdeshell et al. 2008)</DisplayText><record><rec-number>8</rec-number><foreign-keys><key app="EN" db-id="rpv9f5v5cfrzxgesv5bv9sdn0e9dz2vdpzwx" timestamp="0">8</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Howdeshell, K. L.</author><author>Wilson, V. S.</author><author>Furr, J.</author><author>Lambright, C. R.</author><author>Rider, C. V.</author><author>Blystone, C. R.</author><author>Hotchkiss, A. K.</author><author>Gray, L. E., Jr</author></authors></contributors><titles><title>A mixture of five

phthalate esters inhibits fetal testicular testosterone production in the Sprague-Dawley rat in a cumulative, dose-additive manner</title><secondary-title>Toxicological Sciences</secondary-title><alt-title>Toxicol Sci</alt-title><short-title>Toxicological Sciences</short-title></titles><periodical><full-title>Toxicological Sciences</full-title><abbr-1>Toxicol Sci</abbr-1></periodical><alt-periodical><full-title>Toxicological Sciences</full-title><abbr-1>Toxicol Sci</abbr-1></alt-periodical><pages>153-165</pages><volume>105</volume><number>1</number><dates><year>2008</year></dates><isbn>ISSN 1096-6080
EISSN 1096-0929</isbn><accession-num>18411233</accession-num><label>675206</label><urls><related-urls><url>https://heronet.epa.gov/heronet/index.cfm/reference/download/reference_id/675206C3 - 1097,1547,1678,1713,1826,1891,2187,2188,2195,2205,2206,2207,2212,2245,2294,2305,2320,2366,2516</url></related-urls></urls><custom1>true</custom1><electronic-resource-num>10.1093/toxsci/kfn077</electronic-resource-num><language>English</language></record></Cite></EndNote>]. It is therefore plausible that the lack of effect of DEP on fetal survival is related to its low potency as a disruptor of steroidogenesis, although further research would be necessary to evaluate this hypothesis.

There is some support in the epidemiological literature for an association between DEP exposure and reproductive and developmental outcomes. A systematic review of epidemiological literature by our colleagues found *slight* evidence of an association between DEP exposure and male reproductive outcomes in humans. Inconsistent associations were found between DEP exposure and testosterone or AGD despite the relatively high exposure levels, and it was concluded that DEP does not appear to have a strong antiandrogenic effect in humans. There was also some support in the epidemiological literature for an inverse association between DEP exposure and semen quality, although most studies observed no association or a positive association [ADDIN EN.CITE <EndNote><Cite><Author>Radke</Author><Year>2018</Year><RecNum>71</RecNum><DisplayText>(Radke et al. 2018)</DisplayText><record><rec-number>71</rec-number><foreign-keys><key app="EN" db-id="rpv9f5v5cfrzxgesv5bv9sdn0e9dz2vdpzwx" timestamp="0">71</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Radke, Elizabeth G.</author><author>Braun, Joseph M.</author><author>Meeker, John D.</author><author>Cooper, Glinda S.</author></authors></contributors><titles><title>Phthalate exposure and male reproductive outcomes: A systematic review of the human epidemiological evidence</title><secondary-title>Environment International</secondary-title></titles><periodical><full-title>Environ Int</full-title><abbr-1>Environment international</abbr-1></periodical><pages>764-793</pages><volume>121</volume><dates><year>2018</year><pub-dates><date>2018/12/01/</date></pub-dates></dates><isbn>0160-4120</isbn><urls><related-urls><url>http://www.sciencedirect.com/science/article/pii/S0160412018303404</url></related-urls></urls><electronic-resource-num>https://doi.org/10.1016/j.envint.2018.07.029</electronic-resource-num></record></Cite></EndNote>]. Interestingly, systematic review of the epidemiological literature on the female reproductive effects of phthalates found multiple studies that reported an association with early onset of puberty, pregnancy loss, and preterm birth [ADDIN EN.CITE <EndNote><Cite><Author>Radke</Author><Year>2019</Year><RecNum>88</RecNum><DisplayText>(Radke et al. 2019b)</DisplayText><record><rec-number>88</rec-number><foreign-keys><key app="EN" db-id="rpv9f5v5cfrzxgesv5bv9sdn0e9dz2vdpzwx" timestamp="1545342318">88</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Radke, E.</author><author>Glenn, B.</author><author>Braun, J.</author><author>Cooper, G.

</author></authors></contributors><titles><title>Phthalate exposure and female reproductive and developmental effects: a systematic review of the human epidemiological evidence </title><secondary-title>Environmental International</secondary-title></titles><periodical><full-title>Environmental International</full-title></periodical><volume>130</volume><dates><year>2019</year></dates><urls></urls></record></Cite></EndNote>], which are similar to the outcomes seen in the animal studies.

This systematic review was limited to six health outcome categories; however, few studies reported effects for DEP that were not captured by the PECO. Two of the included studies reported endpoints related to postnatal neurological development: [HYPERLINK \l "_ENREF_10" \o "Fujii, 2005 #22"] performed daily reflex response tests on F1 and F2 Sprague-Dawley rat pups and observed no significant differences between groups, whereas [HYPERLINK \l "_ENREF_45" \o "Pereira, 2007 #18"] noted that pups exposed to DEP had sluggish behavior and movement reduction compared to controls but did not provide quantitative data. Additionally, one study by [ADDIN EN.CITE <EndNote><Cite><Author>Pereira</Author><Year>2007</Year><RecNum>17</RecNum><DisplayText>(Pereira et al. 2007d)</DisplayText><record><rec-number>17</rec-number><foreign-keys><key app="EN" db-id="rpv9f5v5cfrzxgesv5bv9sdn0e9dz2vdpzwx" timestamp="0">17</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Pereira, C.</author><author>Mapuskar, K.</author><author>Vaman Rao, C.</author></authors></contributors><titles><title>A two-generation chronic mixture toxicity study of Clophen A60 and diethyl phthalate on histology of adrenal cortex and thyroid of rats</title><secondary-title>Acta Histochemica</secondary-title><alt-title>Acta Histochem</alt-title><short-title>Acta Histochemica</short-title></titles><pages>29-36</pages><volume>109</volume><number>1</number><dates><year>2007</year></dates><isbn>ISSN 0065-1281
EISSN 16180372</isbn><accession-num>17113135</accession-num><label>789165</label><urls><related-urls><url>https://heronet.epa.gov/heronet/index.cfm/reference/download/reference_id/789165C3 -384,1097,1826,2212,2245,2294,2305,2344,2370,2669</url></related-urls></urls><custom1>true</custom1><electronic-resource-num>10.1016/j.acthis.2006.09.008</electronic-resource-num><language>English</language></record></Cite></EndNote>] reported degenerative effects on the adrenal cortex and thyroid after multigenerational exposure to ≤2.85 mg/kg-day of DEP. Although we did not formally evaluate this study, it appears to have been conducted on the same animals used in other studies by this group [ADDIN EN.CITE ADDIN EN.CITE.DATA] and has the same concerns for risk of bias and sensitivity, so it is likely that this finding would be considered *low confidence* if it had been evaluated. Systematic review of emerging outcomes in the epidemiological literature found *slight* evidence of an association between DEP exposure and metabolic effects (insulin resistance and blood glucose/impaired glucose tolerance) [ADDIN EN.CITE <EndNote><Cite ExcludeYear="1"><Author>Radke</Author><Year>2019</Year><RecNum>90</RecNum><DisplayText>(Radke et al.)</DisplayText><record><rec-number>90</rec-number><foreign-keys><key app="EN" db-id="rpv9f5v5cfrzxgesv5bv9sdn0e9dz2vdpzwx" timestamp="1545342467">90</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Radke, E.</author><author>Galizia, A.</author><author>Thayer, K.</author><author>Cooper, G. </author></authors></contributors><titles><title>Phthalate exposure and metabolic effects: a systematic review of the human epidemiological evidence</title><secondary-title>Environmental

International</secondary-title></titles><periodical><full-title>Environmental International</full-title></periodical><dates><year>2019</year></dates><urls></urls><electronic-resource-num>https://doi.org/10.1016/j.envint.2019.04.040</electronic-resource-num></record></Cite><Cite><Author>Radke</Author><Year>2019</Year><RecNum>90</RecNum><record><rec-number>90</rec-number><foreign-keys><key app="EN" db-id="rpv9f5v5cfrzxgesv5bv9sdn0e9dz2vdpzwx" timestamp="1545342467">90</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Radke, E.</author><author>Galizia, A.</author><author>Thayer, K.</author><author>Cooper, G. </author></authors></contributors><titles><title>Phthalate exposure and metabolic effects: a systematic review of the human epidemiological evidence</title><secondary-title>Environmental International</secondary-title></titles><periodical><full-title>Environmental International</full-title></periodical><dates><year>2019</year></dates><urls></urls><electronic-resource-num>https://doi.org/10.1016/j.envint.2019.04.040</electronic-resource-num></record></Cite></EndNote>], and limited support for an association between DEP exposure and neurodevelopmental effects [ADDIN EN.CITE <EndNote><Cite><Author>Radke</Author><Year>Under review</Year><RecNum>89</RecNum><DisplayText>(Radke et al. Under review)</DisplayText><record><rec-number>89</rec-number><foreign-keys><key app="EN" db-id="rpv9f5v5cfrzxgesv5bv9sdn0e9dz2vdpzwx" timestamp="1545342425">89</key></foreign-keys><ref-type name="Journal Article">17</ref-type><contributors><authors><author>Radke, E.</author><author>Nachman, R.</author><author>Braun, J.</author><author>Cooper, G. </author></authors></contributors><titles><title>Phthalate exposure and neuropsychological and behavioral effects: a systematic review and meta-analysis of the human epidemiological evidence</title></titles><dates><year>Under review</year></dates><urls></urls></record></Cite></EndNote>].

This systematic review highlighted several ways in which future studies could provide further insight into the mechanisms and characterization of hazards of DEP exposure. Most of the available studies were conducted at doses that are far greater than doses expected to be relevant to human exposures. The available low dose studies had significant concerns raised during study evaluation and reported findings that were frequently not supported by studies that tested higher dose levels. The recent review of DEP toxicity by [HYPERLINK \l "_ENREF_5" \o "Consumer Product Safety Commission, 2011 #82"] reached a similar conclusion that the studies by Pereira and coauthors had questionable reliability and were not consistent with effects observed in other studies. Additional studies are needed to confirm findings by these groups that have shown dramatic effects following chronic exposure to DEP at low doses.

Additionally, whereas there is little evidence of DEP-mediated effects on testosterone, an interesting finding of this systematic review is that effects on sperm were observed in several studies that exposed peripubertal or adult animals to DEP for longer durations. This finding is suggestive of the effects on Sertoli cells, seminiferous tubules, and germ cell development that have been observed for other phthalates and are thought to be mediated through an androgen-independent mode of action. For DEP, however, the magnitude of effect on sperm parameters was relatively low and varied across the few available studies. Further studies may be warranted to determine the extent to which this mode of action is conserved for DEP, which has the potential to lead to male reproductive effects in the absence of effects on steroidogenesis.

AUTHOR NOTES

Acknowledgements: This manuscript is dedicated to the memory of Raghu Nath, a biologist at the EPA National Center for Environmental Assessment, who contributed to this systematic review of DEP. We would like to acknowledge Anna Chen, Evangela Matthews, Swati Gummadi, Carolyn Gigot, Mefruz Haque, and Andrew Greenhalgh (EPA student services contractors) for their assistance in data extraction and visualization for this systematic review.

Disclaimer: *The views expressed in this article are those of the author(s) and do not necessarily represent the views or policies of the U.S. Environmental Protection Agency.*

Figure 1: (A) Study evaluation criteria and (B) strength of evidence characterization for DIBP animal toxicology studies.

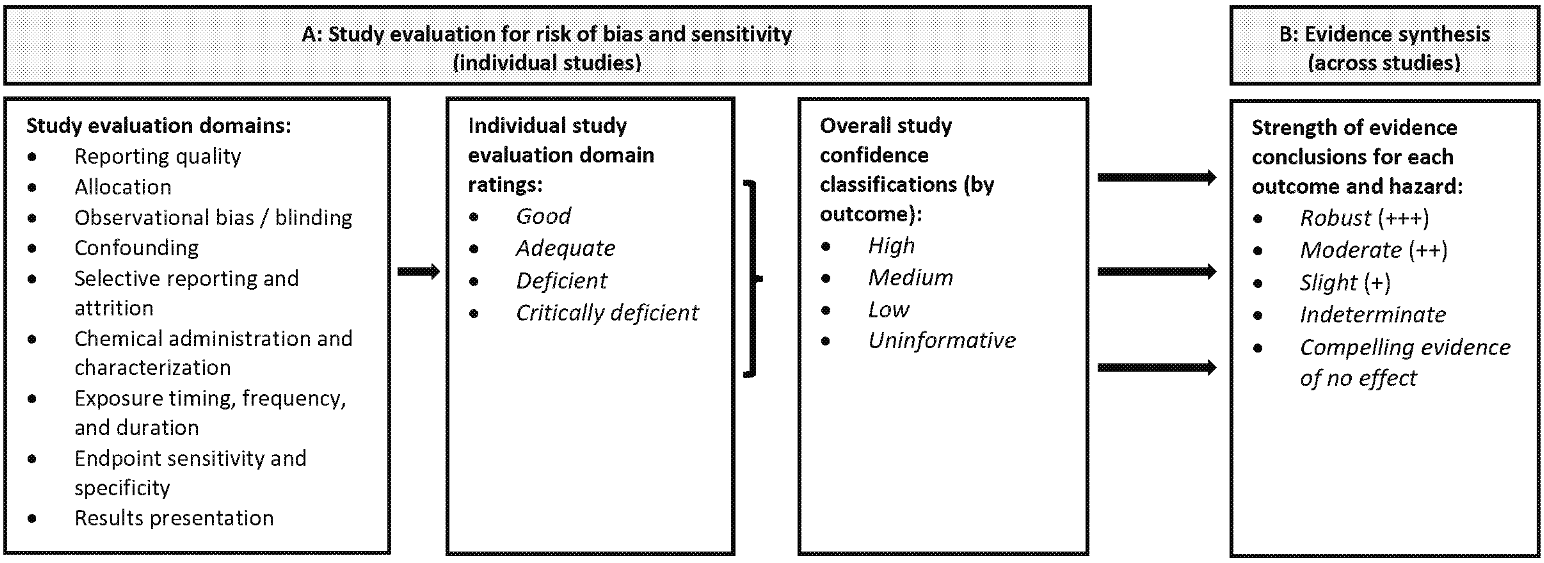
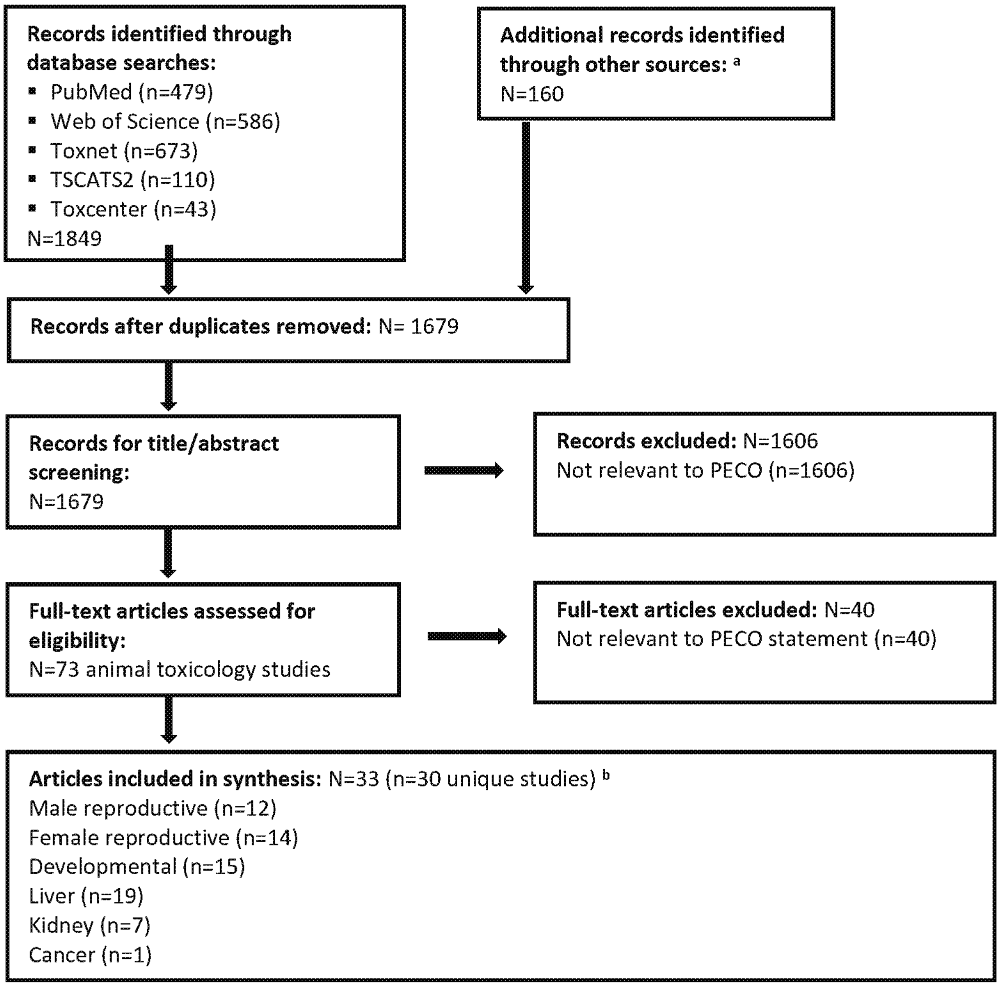


Figure 2: Literature flow diagram for identifying DEP animal toxicology studies.



[a]Other sources consisted of forward and backward searches, searching citations from key references, manual search of citations from key regulatory documents, and references that had been previously identified from an earlier DEP review effort.

[b] Most studies reported data on multiple hazards; see Table 1.

Table 1: List of studies and overall study confidence by outcome[a]

				Male reproductive[a]						Female reproductive[b]							Dev.[c]			Liver				Kidney		
Author (year)	**Species (strain)**	**Exposure life stage and duration**	**Exposure route**	**Testosterone**	**Morphological development**	**Sperm**	**Fertility**	**Organ weight**	**Histopathology**	**Pregnancy outcomes**	**Maternal body weight**	**Organ weight**	**Histopathology**	**Hormones**	**Estrous cyclicity**	**Morphological development**	**Survival**	**Growth**	**Structural alterations**	**Organ weight**	**Histopathology**	**Biochemistry**	**Tumors**	**Organ weight**	**Histopathology**	**Biochemistry**
[HYPERLINK \l "_ENREF_10" \o "Fujii, 2005 #22"]	Rat [Crj:CD (SD)]	Multigenerational study; F0 and F1 each exposed for 10 weeks + mating, gestation, and weaning (~15-17 weeks total)	Diet	H	H	H	H	H	M	H	M	H	M	-	H	H	H	H	-	H	M	H	-	H	M	-
[ADDIN EN.CITE ADDIN EN.CITE.DATA]	Mouse (CD-1)	Multigenerational study (continuous breeding protocol)	Diet	-	-	H	H	H	-	H	-	H	-	-	-	-	H	H	-	H	-	-	-	-	-	-
[HYPERLINK \l "_ENREF_45" \o "Pereira, 2007 #18"]	Rat (Wistar)	Multigenerational study; F0 and F1 each exposed for 100 days + mating, gestation, and weaning (150 days total)]; F2 exposed for 150 days after weaning	Diet	-	-	-	-	-	-	-	-	-	-	-	-	-	-	L	-	L	L	L	-	-	-	-
[HYPERLINK \l "_ENREF_42" \o "Pereira, 2007 #5"]				-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	L	L	L	-	-	-	-
[HYPERLINK \l "_ENREF_41" \o "Pereira, 2007 #38"]				-	-	-	-	L	-	-	-	L	-	-	-	-	-	-	-	-	-	-	-	-	-	-
[HYPERLINK \l "_ENREF_44" \o "Pereira, 2007 #17"]				-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	L	L	L	-	-	-	-

				Male reproductive[a]						Female reproductive[b]							Dev.[c]			Liver				Kidney		
Author (year)	**Species (strain)**	**Exposure life stage and duration**	**Exposure route**	**Testosterone**	**Morphological development**	**Sperm**	**Fertility**	**Organ weight**	**Histopathology**	**Pregnancy outcomes**	**Maternal body weight**	**Organ weight**	**Histopathology**	**Hormones**	**Estrous cyclicity**	**Morphological development**	**Survival**	**Growth**	**Structural alterations**	**Organ weight**	**Histopathology**	**Biochemistry**	**Tumors**	**Organ weight**	**Histopathology**	**Biochemistry**
[ADDIN EN.CITE ADDIN EN.CITE.DATA]	Mouse (CD-1)	GD 6-13	Gavage	-	-	-	-	-	-	-	M	-	-	-	-	-	H	H	-	-	-	-	-	-	-	-
[ADDIN EN.CITE ADDIN EN.CITE.DATA]	Rat (CD)	GD 6-15	Diet	-	-	-	-	-	-	-	H	H	-	-	-	-	H	H	H	-	-	-	-	-	-	-
[HYPERLINK \l "_ENREF_49" \o "Procter & Gamble, 1994 #32"]	Rabbit (New Zealand White)	GD 6-18	Dermal	-	-	-	-	-	-	-	L	H	H	-	-	-	H	H	H	-	-	-	-	-	-	-
[HYPERLINK \l "_ENREF_20" \o "Howdeshell, 2008 #8"]	Rat (Sprague - Dawley)	GD 8-18	Gavage	H	-	-	-	-	-	-	M	-	-	-	-	-	H	-	-	-	-	-	-	-	-	-
[HYPERLINK \l "_ENREF_26" \o "Liu, 2005 #80"]	Rat (Sprague - Dawley)	GD 12-19	Gavage	-	H	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
[HYPERLINK \l "_ENREF_11" \o "Furr, 2014 #42"]	Rat (Sprague - Dawley)	GD 14-18	Gavage	H	-	-	-	-	-	-	L	-	-	-	-	-	M	-	-	-	-	-	-	-	-	-
[HYPERLINK \l "_ENREF_12"	Rat (Sprague - Dawley)	GD 14 - PND 3	Gavage	M	H	-	-	H	-	-	M	-	-	-	-	H	M	H	-	H	-	-	-	H	-	-

Author (year)	Species (strain)	Exposure life stage and duration	Exposure route	Male reproductive[a]						Female reproductive[b]							Dev.[c]			Liver				Kidney		
				Testosterone	Morphological development	Sperm	Fertility	Organ weight	Histopathology	Pregnancy outcomes	Maternal body weight	Organ weight	Histopathology	Hormones	Estrous cyclicity	Morphological development	Survival	Growth	Structural alterations	Organ weight	Histopathology	Biochemistry	Tumors	Organ weight	Histopathology	Biochemistry
\o "Gray, 2000 #10"]																										
[HYPERLINK \l "_ENREF_29" \o "Manservisi, 2015 #48"]	Rat (Sprague -Dawley)	PND 1-181 (females)	Gavage	-	-	-	-	-	-	L	-	-	L	-	-	-	-	L	-		-	-	-		-	-
[HYPERLINK \l "_ENREF_21" \o "Hu, 2016 #47"]				-	-	-	-	-	-	-	-	-	-	-	-	-	-	L	-	-	-	-	-	-	-	-
[HYPERLINK \l "_ENREF_30" \o "Mapuskar, 2007 #24"]	Mouse (Swiss)	2-3 weeks old (females); 90-day exposure	Diet	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	L	L	L	-	-	-	-
[HYPERLINK \l "_ENREF_36" \o "Oishi, 1980 #2"]	Rat (Wistar)	5 weeks old (males); 7-day exposure	Diet	L	-	-	-	M	-	-	-	-	-	-	-	-	-	L	-	M	-	-	-	M	-	-
[HYPERLINK \l "_ENREF_23" \o "Kwack, 2009 #14"]*	Rat (Sprague -Dawley)	5 weeks old (males); 28-day exposure	Gavage	-	-	H	-	L	-	-	-	-	-	-	-	-	-	-	-	H	-	H	-	H	-	H
[HYPERLINK \l "_ENREF_24"	Rat (Sprague -Dawley)	5 weeks old (males); 14-day exposure	Gavage	-	-	-	-	L	-	-	-	-	-	-	-	-	-	-	-	H	-	H	-	H	-	H

Author (year)	Species (strain)	Exposure life stage and duration	Exposure route	Male reproductive[a]						Female reproductive[b]							Dev.[c]			Liver				Kidney		
				Testosterone	Morphological development	Sperm	Fertility	Organ weight	Histopathology	Pregnancy outcomes	Maternal body weight	Organ weight	Histopathology	Hormones	Estrous cyclicity	Morphological development	Survival	Growth	Structural alterations	Organ weight	Histopathology	Biochemistry	Tumors	Organ weight	Histopathology	Biochemistry
\o "Kwack, 2010 #87"]*																										
[HYPERLINK \l "_ENREF_3" \o "Brown, 1978 #72"]	Rat (CD)	Age not reported, but likely pre-pubertal, based on body weight (males and females); 42-day and 112-day exposures	Diet	-	-	-	-	L	L	-	-	L	-	-	-	-	-	-	-	M	L	M	-	M	L	M
[HYPERLINK \l "_ENREF_35" \o "NTP, 1995 #27"]	Mouse (B_6C_3 F_1), Rats (F344/N)	6 weeks old (males and females); 28-day or 104-105-week exposure	Dermal	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	H	H	H	H	H	H	-
[HYPERLINK \l "_ENREF_38" \o "Pereira, 2006 #25"]	Rat (Wistar)	6-7 weeks old (males); 150-day exposure	Diet	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	L	L	L	-	-	-	-
[HYPERLINK \l "_ENREF_60" \o "Sonde, 2000 #19"]	Rat (Sprague - Dawley)	7 weeks old (males); 120-day exposure	Drinking water	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	L	-	L	-	-	-	-
[HYPERLINK \l "_ENREF_39" \o "Pereira, 2006 #37"]	Rat (Wistar)	6-7 weeks old (females); 150-day exposure	Diet	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	L	-	-	-	-
[HYPERLINK \l "_ENREF_40"	Rat (Wistar)	7-8 weeks old (males and	Diet	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	L	L	-	-	-	-

Author (year)	Species (strain)	Exposure life stage and duration	Exposure route	Male reproductive[a]						Female reproductive[b]							Dev.[c]			Liver				Kidney		
				Testosterone	Morphological development	Sperm	Fertility	Organ weight	Histopathology	Pregnancy outcomes	Maternal body weight	Organ weight	Histopathology	Hormones	Estrous cyclicity	Morphological development	Survival	Growth	Structural alterations	Organ weight	Histopathology	Biochemistry	Tumors	Organ weight	Histopathology	Biochemistry
\o "Pereira, 2006 #23"]		females); 150-day exposure																								
[HYPERLINK \l "_ENREF_46" \o "Pereira, 2008 #34"]	Rat (Wistar)	7-8 weeks old (males); 150-day exposure	Diet	L	-	-	-	L	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
[HYPERLINK \l "_ENREF_47" \o "Pereira, 2008 #35"]	Rat (Wistar)	7-8 weeks old (males); 150-day exposure	Diet	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	L	L	L	-	-	-	-
[HYPERLINK \l "_ENREF_32" \o "Moody, 1978 #16"]	Rat (F334)	Age not reported (males); 21-day exposure	Diet	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	M	-	M	-	-	-	-
[HYPERLINK \l "_ENREF_57" \o "Shiraishi, 2006 #30"]	Rat (Sprague - Dawley	8 weeks old (males and females); 28-day exposure	Gavage	H	-	M	-	L	M	-	-	H	M	H	M	-	-	-	-	H	M	M	-	H	M	-
[HYPERLINK \l "_ENREF_59" \o "Sinkar, 2007 #36"]	Rat (Wistar)	12 weeks old (males and females); 180-day exposure	Drinking water	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	L	L	-	-	-	-

*Kwack et al. tested both DEP and MEP. The remaining studies tested DEP.

[a]High confidence (H), Medium confidence (M), Low confidence (L). Dash (-) indicates endpoints that were not included in a study.

[b]Male reproductive endpoints: Testosterone (testicular production or level measured in testis or serum), morphological development (AGD, nipple retention, time to puberty), sperm (sperm counts, motility, morphology), fertility (copulation and ability to produce offspring), organ weights (testis, epididymides, prostate, seminal vesicles), histopathology (general histopathological evaluations of male reproductive organs)
[c]Female reproductive endpoints: Pregnancy outcomes (copulation, litter size, gestation length), maternal body weight (body weight gain during gestation or lactation), organ weight (uterus, vagina, ovary), histopathology (general histopathological evaluations of female reproductive organs), hormones (any measurement of reproductive hormones), estrous cyclicity, morphological development (AGD, time to puberty),
[d]Developmental endpoints: Survival (fetal viability, fetal mortality, resorptions, pre- or post-implantation loss, postnatal survival), growth (pre- or postnatal body weight), structural alterations (external, skeletal, or visceral malformations or variations)

Table 2: Evidence profiles table for male reproductive outcomes.

Male reproductive effects						
	Outcome	**Available Studies**	**Factors that increase confidence**	**Factors that decrease confidence**	**Strength of evidence conclusion for outcome**	**Strength of evidence conclusion for overall hazard**
Gestational or postnatal exposure (including F1 and F2 offspring from multigenerational studies)	**Testosterone**	**High Confidence**: Howdeshell et al. 2008 Furr et al. 2014 **Medium Confidence:** Gray et al. 2000	• Consistency • Minimal concern for bias and sensitivity	• Few studies	○○○ INDETERMINATE No significant effects on fetal testosterone production at GD 18 (Howdeshell et al. 2008, Furr et al. 2014) or serum testosterone levels at PND 3 (Gray et al. 2000) were observed following gestational exposure.	⊕⊕○ MODERATE Based on moderate evidence for adverse effects on sperm and decreased testosterone in adult exposure studies.
	Male morphological development	**High Confidence:** Fujii et al. 2005 Gray et al 2000 Liu et al. 2005	• Consistency • Minimal concern for bias and sensitivity	• Few studies	○○○ INDETERMINATE There were no DEP-related effects on nipple retention or AGD (Gray et al. 2000, Liu et al. 2005) or on the age of preputial separation (Fujii et al. 2005, Gray et al. 2000).	
	Reproductive organ weights	**High Confidence**: Fujii et al. 2005 Gray et al. 2000	• Consistency • Minimal concern for bias and sensitivity	• Few studies	○○○ INDETERMINATE There were no effects on male reproductive organ weights following gestational exposure, except for a decrease in absolute prostate weight and increase in relative seminal vesicle weight in F1 weanlings (Fujii et al. 2005).	

Peripubertal or adult exposure (including F0 and F1 parental animals from multigenerational studies)	**Testosterone**	**High Confidence:** Fujii et al. 2005 Shiraishi et al. 2006 **Low Confidence**: Oishi and Hiraga 1980 Pereira et al. 2008b	• Consistency in the direction of effect across three studies	• Few studies • Low precision in the high confidence study by Fujii et al. 2005 • Concerns for bias and sensitivity in studies by Oishi and Hiraga 1980 and Pereira et al. 2008b	⊕○○ SLIGHT Significantly decreased testosterone was observed in rats exposed as adults/young adults in the studies by Fujii et al. 2005, Pereira et al. 2008b, and Oishi and Hiraga 1980, although Fujii et al. 2005 tested a relatively small sample size and had high variability, and the latter two studies are considered *low confidence* for this outcome. No effects on testosterone were observed in adult male rats after a 28-day exposure (Shiraishi et al. 2006).
	Sperm	**High Confidence**: Fujii et al. 2005 RTI International 1984 Kwack et al. 2009 **Medium Confidence:** Shiraishi et al. 2006	• Minimal concern for bias and sensitivity • Effect size • Biological plausibility	• Few studies • Unexplained inconsistency	⊕⊕○ MODERATE Decreased sperm number, increased incidence of abnormal sperm, and/or decreased sperm motility were observed in multiple studies that exposed adult rats or mice (Fujii et al. 2005, Kwack et al. 2009, RTI International 1984), although effects differed somewhat between studies and were not consistently observed. Shiraishi et al. 2006 used a similar study design as Kwack et al. 2009 but did not observe any effects.
	Fertility	**High Confidence**: Fujii et al. 2005	• Consistency	• Few studies	○○○

		RTI International 1984	• Minimal concern for bias and sensitivity		INDETERMINATE No effects on fertility in mating pairs were observed in two multigenerational reproductive studies.	
	Reproductive organ weights	**High Confidence:** RTI International 1984 Fujii et al. 2005 **Low Confidence:** Brown et al. 1978 Shiraishi et al. 2006 Kwack et al. 2009 Kwack et al. 2010 Pereira et al. 2007b Pereira et al. 2008a		• Unexplained inconsistency • Concerns for bias and sensitivity	○○○ INDETERMINATE Absolute organ weights (testes, epididymides, prostate, seminal vesicles) were generally not affected in two *high confidence* studies in mice (RTI International 1984) and rats (Fujii et al. 2005). Effects on relative testis weight were inconsistent across the remaining *low confidence* studies, although this is considered a less reliable measurement compared to absolute testis weight.	
	Histopathology	**Medium Confidence:** Fujii et al. 2005 Shiraishi et al. 2006 **Low Confidence:** Brown et al. 1978		• Few studies • Quantitative data not reported in any study	○○○ INDETERMINATE No effects on histopathology of male reproductive organs were observed in three studies.	

Table 3: Evidence profiles table for female reproductive outcomes.

Female reproductive effects					
Outcome	**Available studies**	**Factors that increase confidence**	**Factors that decrease confidence**	**Strength of evidence conclusion for outcome**	**Strength of evidence conclusion for overall hazard**
Pregnancy outcomes	**High Confidence:** Fujii et al. 2005 RTI International 1984 **Low Confidence:** Manservisi et al. 2015	• Minimal concern for bias and sensitivity in most studies	• Few studies • Small magnitude of effect in *high confidence* studies	⊕◯◯ SLIGHT Statistically significantly decreased gestation length (Fujii et al. 2005) and decreased litter size (RTI International 1984) were observed in F1 parental females in two *high* confidence studies, and increased litter size was observed in one *low* confidence study (Manservisi et al. 2015). Otherwise, no effects were observed.	⊕◯◯ SLIGHT Based on few reported effects across studies, including effects on gestation length, litter size, and organ weights or histopathology.
Maternal body weight	**High Confidence***:* NTP 1988 **Medium Confidence***:* Howdeshell et al. 2008 Hardin et al. 1987 Gray et al. 2000 Fujii et al. 2005 **Low Confidence:** Furr et al. 2014 Procter & Gamble 1994	• Few concerns for bias and sensitivity in two studies that observed effects	• Unexplained inconsistency	◯◯◯ INDETERMINATE NTP 1988 reported a trend towards decreased maternal body weight gain (corrected for gravid uterine weight), whereas Fujii et al. 2005 reported increased maternal body weight gain in F0 females. Otherwise, no effects on maternal body weight gain were observed in rats, mice, or rabbits.	
Reproductive organ weights	**High Confidence***:* Fujii et al. 2005		• Concerns for bias and sensitivity in one out of two studies that observed effects	⊕◯◯ SLIGHT Decreased absolute and relative uterine weights was observed in F1 and F2 weanling rats in a *high*	

	RTI International 1984 Procter & Gamble 1994 NTP 1988 Shiraishi et al. 2006 **Medium Confidence:** Brown et al. 1978 **Low Confidence***:* Pereira et al. 2007a			*confidence* two-generation study in rats (Fujii et al. 2005), although the effect was not observed in adult F1 animals so may be transient. A significant decrease in relative ovary weights was reported in a *low confidence* study in F0 and F1 adult rats (Pereira et al. 2007a). Otherwise, no organ weight changes were observed.	
Histopathology	**High Confidence***:* Procter & Gamble 1994 **Medium Confidence:** Fujii et al. 2005 Shiraishi et al. 2006 **Low Confidence***:* Manservisi et al. 2015		• Concerns for bias and sensitivity in the only study that observed effects	⊕◯◯ SLIGHT A reduction in the size of the lobular structures of the mammary gland was observed in a *low confidence* study in parous F1 female rats (Manservisi et al. 2015). Otherwise, no histopathological changes were observed in ovaries, uteri, vaginas, or mammary glands.	
Hormones	**High Confidence**: Shiraishi et al. 2006	• Minimal concern for bias and sensitivity	• Single study	◯◯◯ INDETERMINATE No effects were observed on steroid hormone or gonadotropin levels in females exposed to DEP as adults (Shirashi et al. 2006).	
Estrous cyclicity	**High Confidence**: Fujii et al. 2005	• Minimal concern for bias and sensitivity	• Single study	◯◯◯ INDETERMINATE No effects were observed on estrous cyclicity in F0 or F1 females in a two-generation study (Fujii et al. 2005).	

Female morphological development	**High Confidence**: Fujii et al. 2005 Gray et al. 2000	• Minimal concern for bias and sensitivity	• Single study	◯◯◯ INDETERMINATE A statistically significant decrease in the age at vaginal opening was observed in F1 rats in a two-generation study, likely related to decreased growth. No DEP-related effects on AGD were observed in F1 or F2 animals in this study (Fujii et al. 2005).	

Table 4: Evidence profiles table for developmental outcomes.

Developmental effects					
Outcome	**Available studies**	**Factors that increase confidence**	**Factors that decrease confidence**	**Strength of evidence conclusion for outcome**	**Strength of evidence conclusion for overall hazard**
Fetal survival	**High Confidence***:* Fujii et al. 2005 Procter and Gamble 1994 Hardin et al. 1987 Howdeshell et al. 2008 RTI International 1984 NTP 1988 **Medium confidence***:* Furr et al. 2014 Gray et al. 2000	• Minimal concerns for bias and sensitivity in most studies	• Effects observed in only one study	◯◯◯ INDETERMINATE A continuous breeding study reported decreased live F2 pups at birth (RTI International 1984). No other studies reported dose-related effects on fetal survival.	⊕⊕◯ MODERATE Based primarily on the consistent reduction in postnatal growth observed across gestational and early postnatal exposure studies.
Postnatal survival	**High Confidence***:* Fujii, et al. 2005 Hardin et al. 1987 **Low Confidence:** Manservisi et al. 2015	• Minimal concerns for bias and sensitivity in most studies	• Unexplained inconsistency	◯◯◯ INDETERMINATE Decreased postnatal survival of F2 pups was observed in the *low confidence* study by Manservisi et al. (2015), and there was a nonsignificant trend towards decreased survival of F1 but not F2 pups in the high *confidence* study by Fujii et al. 2005. Otherwise, no effects were observed.	
Fetal growth	**High Confidence** Fujii et al. 2005 RTI International 1984 NTP 1988 Hardin et al. 1987 Procter & Gamble 1994 Gray et al. 2000	• Minimal concerns for bias and sensitivity		◯◯◯ INDETERMINATE All studies that assessed prenatal growth observed that DEP had little to no effect on fetal body weight or body weight at birth in rats, mice or rabbits.	

Postnatal growth	**High Confidence** Fujii et al. 2005 RTI International 1984 Hardin et al. 1987 Gray et al. 2000 **Low Confidence**: Pereira and Rao 2007 Manservisi et al. 2015 Oishi and Hiraga 1980 Hu et al. 2016	• Consistency across studies with longer exposure durations	• Concerns for risk of bias and sensitivity in some studies	⊕⊕⊕ ROBUST Decreased postnatal body weights were observed in offspring in the *high confidence* multigenerational exposure studies by Fujii et al. 2005 and RTI International 1984, including effects in both F1 and F2 generations in the study by Fujii et al. 2005, and in the *low confidence* studies by Pereira and Rao 2007, Manservisi et al. 2015, and Hu et al. 2016. No effects on growth were observed following shorter duration gestational exposures (Hardin et al. 1987, Gray et al. 2000) or peripubertal exposure (Oishi and Hiraga 1980).	
Fetal structural alterations	**High Confidence**: NTP 1988 Procter & Gamble 1994	• Exposure-response gradient in the study by NTP 1988 • Minimal concern for bias and sensitivity	• Few studies	⊕◯◯ SLIGHT A dose-related increase in the percentage of fetuses with a rudimentary or supernumerary rib was observed in rats exposed during gestation (NTP 1988). Findings in rabbits (Procter & Gamble 1994) including fused ribs, missing lumbar, coccygeal vertebrae, hernia umbilicalis, and incurved ribs were not considered to be related to dose.	

Table 5: Evidence profiles table for effects on liver.

Liver effects					
Outcome	**Available studies**	**Factors that increase confidence**	**Factors that decrease confidence**	**Strength of evidence conclusion for outcome**	**Strength of evidence conclusion for overall hazard**
Organ weight	**High Confidence***:* Fujii et al. 2005 Gray et al. 2000 NTP 1995 RTI International 1984 Kwack et al. 2009 Kwack et al. 2010 Shiraishi et al. 2006 **Medium Confidence** Moody and Reddy 1978 Oishi and Hiraga 1980 Brown et al. 1978 **Low Confidence:** Pereira and Rao 2006a Pereira et al. 2006 Pereira and Rao 2007 Pereira et al. 2007b Pereira et al. 2007c Pereira et al. 2008c Mapuskar et al. 2007 Sonde et al. 2000	• Consistency across most higher dose studies • Biological plausibility	• Inconsistent findings in low dose studies • Low magnitude of effect	⊕⊕◯ MODERATE Dose-related increases in relative liver weight were observed in most studies that used higher dose levels of DEP, with statistically significant changes only observed at the highest doses tested (Fujii et al. 2005, Brown et al. 1978, NTP 1995, Moody and Reddy 1978, RTI International 1984, Oishi and Hiraga 1980). No effects on liver weight were observed in four rat studies (Gray et al. 2000, Kwack et al. 2009, 2010; Shirashi et al. 2006) and effects on liver weight were inconsistent across a group of *low confidence* low dose studies (Pereira and Rao 2006a, Pereira et al. 2006, Pereira and Rao 2007, Pereira et al. 2007b, Pereira et al. 2007c, Pereira et al. 2008c, Mapuskar et al. 2007, Sonde et al. 2000).	⊕⊕◯ MODERATE Based on evidence of increased liver weight, histopathological effects, and biochemical changes that are indicative of hepatic effects. However, evidence for histopathological and biochemical effects was primarily found in low dose studies that had concerns for bias and sensitivity.
Histopathology	**High Confidence***:* NTP 1995 **Medium confidence***:* Fujii et al. 2005 Shiraishi et al. 2006	• Biological plausibility	• Unexplained inconsistency • Quantitative results are generally not provided	◯◯◯ INDETERMINATE Reports of intracellular vacuolations, degenerative changes in centrilobular and	

	Low confidence: Mapuskar et al. 2007 Sinkar and Rao 2007 Brown et al. 1978 Pereira et al. 2006 Pereira and Rao 2007 Pereira et al. 2007b Pereira et al. 2007c Pereira et al. 2008c Pereira and Rao 2006a		• Concerns for bias and sensitivity in low dose studies that observed effects	periportal areas, and necrosis were observed in *low confidence* low dose studies ranging from 0.57 mg/kg-day to 6.25 mg/kg-day, although these studies did not provide quantitative data (Pereira et al. 2006, Pereira and Rao 2007, Pereira et al. 2007b, Pereira et al. 2007c, Pereira et al. 2008c, Pereira and Rao 2006a, Sinkar and Rao 2007, Mapuskar et al. 2007). Milder effects (NTP 1995, or no effects (Fujii et al. 2005, Brown et al. 1978) on liver histopathology were observed in studies that used higher dose levels.	

Table 6: Evidence profiles table for effects on kidney.

Kidney effects					
Outcome	**Available studies**	**Factors that increase confidence**	**Factors that decrease confidence**	**Strength of evidence conclusion for outcome**	**Strength of evidence conclusion for overall hazard**
Organ weight	**High Confidence**: Fujii et al. 2005 Kwack et al. 2009 Kwack et al. 2010 NTP 1995 Shiraishi et al. 2006 Gray et al. 2000 **Medium confidence:** Oishi and Hiraga 1980 Brown et al. 1978	• Minimal concern for bias • Biological plausibility	• Unexplained inconsistency	○○○ INDETERMINATE Inconsistent findings were reported for changes in kidney weight. Increases, decreases, or no effect in relative and absolute kidney weight were reported following oral or dermal DEP exposures in rats or mice, with no clear pattern of effect.	○○○ INDETERMINATE Based on inconsistent effects on kidney weight across studies, limited biochemical findings, and lack of histopathological findings.
Histopathology	**High Confidence**: NTP 1995 **Medium Confidence:** Fujii et al. 2005 Shirashi et al. 2006 **Low Confidence:** Brown et al. 1978	• Consistency	• Few studies	○○○ INDETERMINATE No treatment-related histopathological lesions in the kidney were reported in rats or mice following DEP exposure.	
Biochemistry	**High Confidence** Kwack et al. 2009 Kwack et al. 2010 **Medium Confidence:** Brown et al. 1978	• Biological plausibility	• Few studies	○○○ INDETERMINATE Serum calcium levels were found to be increased in rats, with no effect on other biochemical parameters related to kidney injury (Kwack et al., 2009, 2010). Findings for urinary cell excretion were inconsistent (Brown et al. 1978).	

Table 7: Evidence profiles table for cancer.

Cancer					
Outcome	**Available studies**	**Factors that increase confidence**	**Factors that decrease confidence**	**Strength of evidence conclusion for outcome**	**Strength of evidence conclusion for overall hazard**
Tumors	**High Confidence** NTP 1995	• Biological plausibility	• Single study	○○○ INDETERMINATE Increased incidence of liver neoplasms was reported in male and female mice following a 2-year bioassay. A dose-related trend was observed in adenomas and carcinomas males; however, the increase was within the 2-year historical norms. In females, no dose response was observed.	○○○ INDETERMINATE

[ADDIN EN.REFLIST]